Battling Illness With Body Proteins

Recombinant DNA technology has enabled medical researchers to manufacture several human proteins that can bolster the body's natural defenses against disease

BY MARY MURRAY

Thirteen years have passed since medical researchers Stanley Cohen and Herbert Boyer first pasted frog genes into the plasmids of *Escherichia coli* bacteria. As hoped, the bacteria were able to manufacture the frog RNA encoded in the genes. It was the first step in learning how to give bacteria the genetic ability to produce animal proteins.

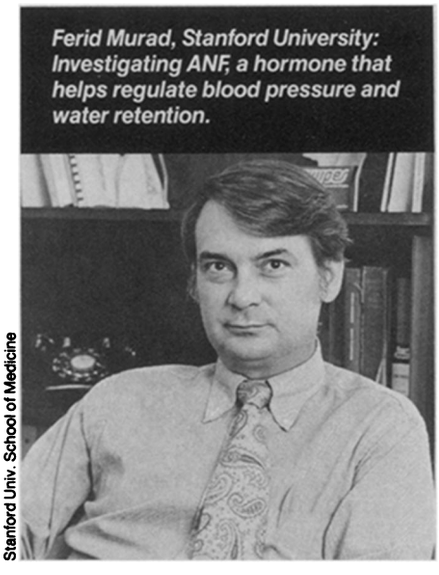
Very quickly, the medical community realized that Cohen of Stanford University and Boyer of the University of California at San Francisco had come up with the key to a new biological technology — recombinant DNA — which could induce bacteria to "clone" large amounts of specific proteins. And almost as quickly, there began widespread, unbridled speculation about the medically useful human proteins that could be cloned. It seemed scientists could make vaccines against diseases ranging from cholera to malaria, brain opiates that could replace artificial painkillers and enough cancer-fighting cells to eradicate many forms of the disease.

It's no wonder that, to date, recombinant DNA technology has not lived up to such lofty expectations. Nevertheless, medical researchers have been able to develop a respectable range of pharmaceuticals using Cohen and Boyer's bacterial technique.

By 1982, the U. S. Food and Drug Administration (FDA) had approved for general use the first gene-cloned medical product: human insulin, which diabetics must take in drug form and which previously came only from cow and pig pancreases. And within the last two years, the FDA has approved recombinant human growth hormone, to treat growth-hormone-deficient children of short stature; alpha interferon, to break down tumors in people with hairy cell leukemia; and a vaccine against hepatitis B.

Medical researchers now are at work testing other potentially therapeutic human proteins that can be manufactured from recombinant DNA. Clinical trials are under way on new forms of cancer-fighting enzymes, on proteins capable of breaking up blood clots and on hormones that lower blood pressure and reduce water retention. None of these is a cure for any illness, but all promise to be strong defensive weapons that could slow or halt the progress of disease.

Recently, a group of these researchers gathered at Stanford University's medical school to report on their latest work, providing a convenient snapshot of the present state of recombinant pharmaceuticals.



Ferid Murad, Stanford University: Investigating ANF, a hormone that helps regulate blood pressure and water retention.

Stanford Univ. School of Medicine

They described, for example, two of the new recombinant proteins designed to help heart patients: atrial natriuretic factor (ANF), a hormone capable of reducing blood pressure and water retention in people suffering from congestive heart failure and hypertension; and tissue plasminogen activator (tPA), a clot-dissolving enzyme that can reduce tissue damage after sudden heart attacks.

ANF, normally made by the heart, was cloned in 1983 and is still in the "very preliminary" stages of investigation, Ferid Murad told some 100 scientists at the symposium. Murad, acting chairman of Stanford's Department of Medicine, says that so far the hormone has been tested on only a few dozen patients, but it already has demonstrated its ability to lower blood pressure and reduce water retention. Murad himself has given ANF to 10 patients with congestive heart failure, and most of them benefited, he says.

"We have found it does improve the excretion of salt and water in most cases," Murad told SCIENCE NEWS. "The question is, can it be useful in therapy over the long term?"

Murad recently has found that one of the ways ANF operates is by relaxing the muscles in blood vessels, thereby helping to reduce blood pressure. ANF also appears to enhance the movement of water and sodium through the body, increasing urine volume and the excretion of salt, he reports.

Murad has observed that ANF also affects the kidneys, lungs, liver, brain, intestines and testes. Laboratory experiments have shown, for example, that it can raise or lower levels of the hormone testosterone in men, depending on how much is

administered. And it appears ANF may affect the intestines in such a way as to induce diarrhea. Some of these noncardiovascular effects may turn out to be potential new uses for recombinant ANF, Murad says; others may turn out to be serious side effects. "Where this peptide is going is a little bit unpredictable," he says.

Tissue plasminogen activator, a protein produced in the linings of the heart and blood vessels, is farther along than ANF in clinical trials (SN: 3/10/84, p.151, 4/13/85, p.229). Since it first was cloned in 1983, tPA has shown great promise in tests on some 2,500 heart attack patients worldwide, and it is expected to get FDA approval in 1987.

In most of the trials, tPA has broken up clots significantly faster and more frequently than the traditional clot-busting medicines: streptokinase, a bacterial enzyme; and urokinase, an enzyme found in the urine, Burton E. Sobel reported at the symposium. Sobel has directed tPA trials at the Washington University School of Medicine in St. Louis, where he is director of the cardiovascular division.

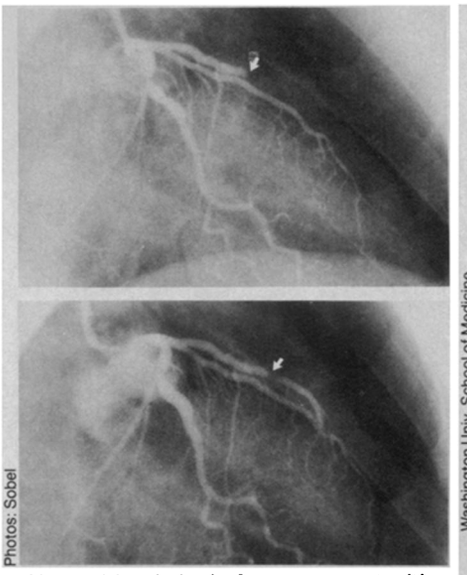
So far, Sobel says, tPA also appears safer than its predecessors because it specifically targets blood clots and does not break down clotting substances elsewhere in the bloodstream. This has reduced to a minimum the threat of excess bleeding. And tPA apparently does not trigger the formation of antibodies, as do streptokinase and urokinase.

At this point, Sobel says, the challenge is to find ways to get tPA to the patients quickly enough to do some good. Unless clots can be dissolved within one to three hours after a heart attack, he explains, heart tissue can suffer irreparable damage.

Sobel is looking into the possibility of allowing some people to inject themselves with tPA as soon as they realize they've had a heart attack, in order to save the time it takes to get to the hospital. To make this possible, he and his colleagues first had to find a way to speed up muscle absorption of tPA, which usually takes 60 to 90 minutes. (In the hospital, the drug is given intravenously, but this would be difficult for patients to do themselves.) So they developed an "absorption enhancer," which puts intramuscularly injected tPA to work within two to five minutes.

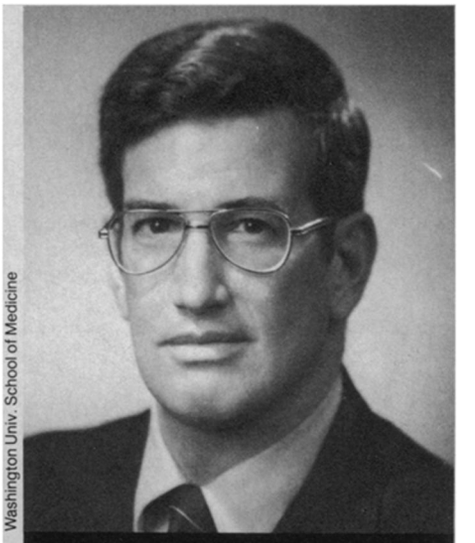
Even if self-injection can be made to work quickly, however, it would have to be carefully supervised, Sobel says. "Obviously, you're not going to arm people with fibrolytic activators [clot dissolvers] that they could use with abandon," he says. "It's going to require . . . very strict control."

Some physicians have made tPA act faster by increasing dose levels. But Sobel warns that if the doses become too large, the protein is likely to persist in the patient's bloodstream and raise the possibility of excess bleeding. Although tPA has caused only minimal bleeding so far, it is not "absolutely clot-specific," Sobel says, and with very large doses, bleeding is likely to occur.



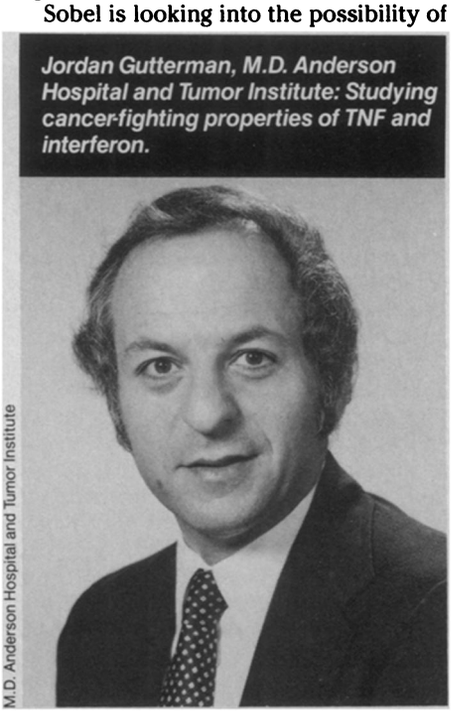
Photos: Sobel

Above, blood clot before treatment with tPA. Below, same clot dissolving 30 minutes after tPA reaches it.



Washington Univ. School of Medicine

Burton E. Sobel, Washington University: Finding ways to use tPA quickly against heart-damaging clots.



M.D. Anderson Hospital and Tumor Institute

Jordan Gutterman, M.D. Anderson Hospital and Tumor Institute: Studying cancer-fighting properties of TNF and interferon.

Researchers say it is unlikely that any of the recombinant pharmaceuticals will be totally free of side effects. In each case, the challenge is to identify the problems and find out whether they outweigh the drug's potential benefits. The story of tumor necrosis factor (TNF), one of the recombinant anticancer drugs, illustrates how researchers must contend with potentially devastating side effects.

As Jordan U. Gutterman of the M. D. Anderson Hospital and Tumor Institute in Houston reported at the Stanford symposium, long-term treatment with low doses of TNF appears to kill cancer cells, both in cell cultures and in animals, without toxic effects on healthy tissue.

However, it appears that TNF also may be capable of causing severe weight loss, fever, anemia, shock and even death. Scientists first discovered this possibility in August 1985, when tests revealed that TNF is identical to another body protein called cachectin, which fights bacterial infections and chronic diseases. When cachectin is unable to eradicate the disease — as in the case of a patient fighting a losing battle against cancer or a massive parasitic infection — it can accumulate until it has a toxic effect on the body. This often fatal condition, called cachexia, is marked by rapid wasting of body tissues.

"The two proteins [TNF and cachectin] are, in fact, identical," Anthony Cerami, head of the medical biochemistry laboratory at Rockefeller University in New York City, told scientists at the symposium.

Cachectin kills cells by turning off the messenger RNA responsible for producing proteins that break down fats and, at the same time, by creating a great demand for energy, Cerami explains. This leads to a "futile cycle," in which cells attempt to bring in great quantities of the body's stored glucose to supply the needed energy; when there is not enough glucose to meet the demand, the cachexic patient's tissues begin to break down.

If the protein is to be used as a cancer treatment, researchers first must find a proper dose — small enough to avoid cachexia-like effects, yet large enough to kill tumor cells. Unfortunately, Cerami says, it is difficult to tell exactly how much of the protein is detrimental because it is difficult to find in the body. "We have found it extremely difficult to measure in disease states, even when we suspect it's

playing a major role," he says.

To date, clinical trials using very low but effective doses of recombinant TNF have not caused weight loss, anemia or shock, according to Gutterman. The only side effects that have turned up so far in these patients are fever, fatigue, some decrease in blood pressure and, when the protein is administered by intramuscular rather than intravenous injection, some skin inflammation, Gutterman reports.

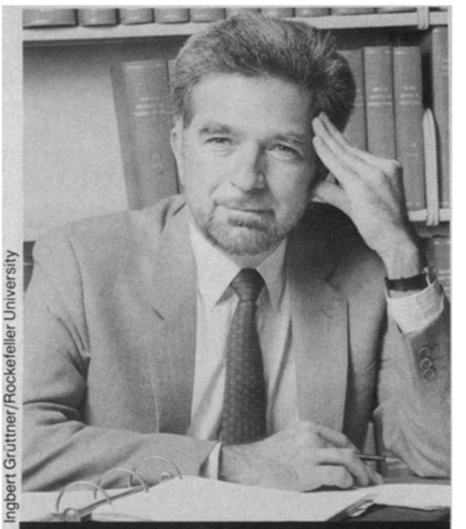
Another immune system agent that may have similar but less severe effects is interleukin-1 (IL-1), a protein cloned in 1984. IL-1 is related functionally to interleukin-2 (IL-2), which has been used to fight cancer (SN: 12/13/86, p.373). IL-1 stimulates the production of IL-2 and helps IL-2 bind to cell membranes. In the case of IL-1, however, the bulk of research has been aimed not at using it against cancer but rather at understanding its natural role in the immune system.

At the symposium, Charles Dinarello of the Tufts University School of Medicine in Boston described laboratory tests on rabbits that have shown how IL-1 fosters the body's response to trauma or infection by inducing fever, causing inflammation and increasing the need for deep sleep. In cases of extreme infection, IL-1 can go farther, causing prolonged fever and inflammation and muscle deterioration. IL-1 may be at work in arthritis, Dinarello says, because abnormally high quantities of the protein have been found in the joint spaces of arthritis patients.

"It's clear that IL-1 and cachectin share biological activities, and yet they're totally distinct molecules," Dinarello says. "The question is, why wasn't there any evolutionary pressure to get rid of these molecules? Why do [our bodies] continue to produce them?"

In addition to his recent work on TNF, Gutterman has continued his long-term studies of alpha interferon, the oldest of the anticancer recombinant proteins. Cloned in late 1979 and approved last summer for use against hairy cell leukemia, alpha interferon appears to cause at least partial remission 75 to 90 percent of the time, not only in patients with hairy cell leukemia (HCL), a rare disease that affects mainly men, but also in patients with chronic myelogenous leukemia (CML), a genetically caused cancer that usually lingers for more than three years before killing the patient (SN: 4/26/86, p.262), Gutterman reports. Some patients have had side effects, however, including fatigue, depression, weight loss, cough, postnasal drip and body aches, he says.

Gutterman has stopped treating some HCL patients whose cancers have gone into total remission. He says it is too soon to know whether they will be able to sustain remission, but he expects the cancers to recur eventually. "It's my best guess that we're not curing the disease," he says. "The patient will not die [of HCL], but it's not clear whether we're going to be able to eradicate the disease with the alpha interferon alone."

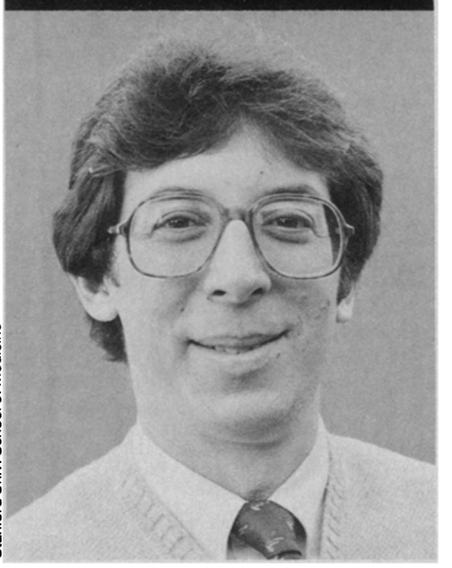


Ingbert Grüttner/Rockefeller University

Anthony Cerami, Rockefeller University: Found that TNF, the anticancer protein, and cachectin, a sometimes destructive protein, are identical.

Alpha interferon has been less effective against other forms of cancer. It has produced remission rates of only 20 to 30 percent in patients with renal cell carcinoma, the most prevalent form of kidney cancer, and in those with multiple myeloma, a form of bone marrow cancer, Gutterman says. Against lung, colon and breast cancer, alpha interferon has been mostly ineffective, bringing about remission in less than 10 percent of patients, he says.

Ron G. Rosenfeld, Stanford University: Studying uses for human growth hormone.



Stanford Univ. School of Medicine

More recently, he has begun testing gamma interferon — a simpler and less understood protein — on renal cell carcinoma and on CML. He reports that so far, six of his 26 CML patients have gone into complete remission.

Gutterman suggests that alpha and gamma interferon might best be used together, as long as care is taken to make sure they do not build up overlapping side effects. Perhaps, he says, the two proteins could be given sequentially. He also suggests that both forms of interferon might work well in combination with TNF.

"I'm very optimistic that over the next several years, using these new proteins, we're going to take very significant strides in medicine," Gutterman says.

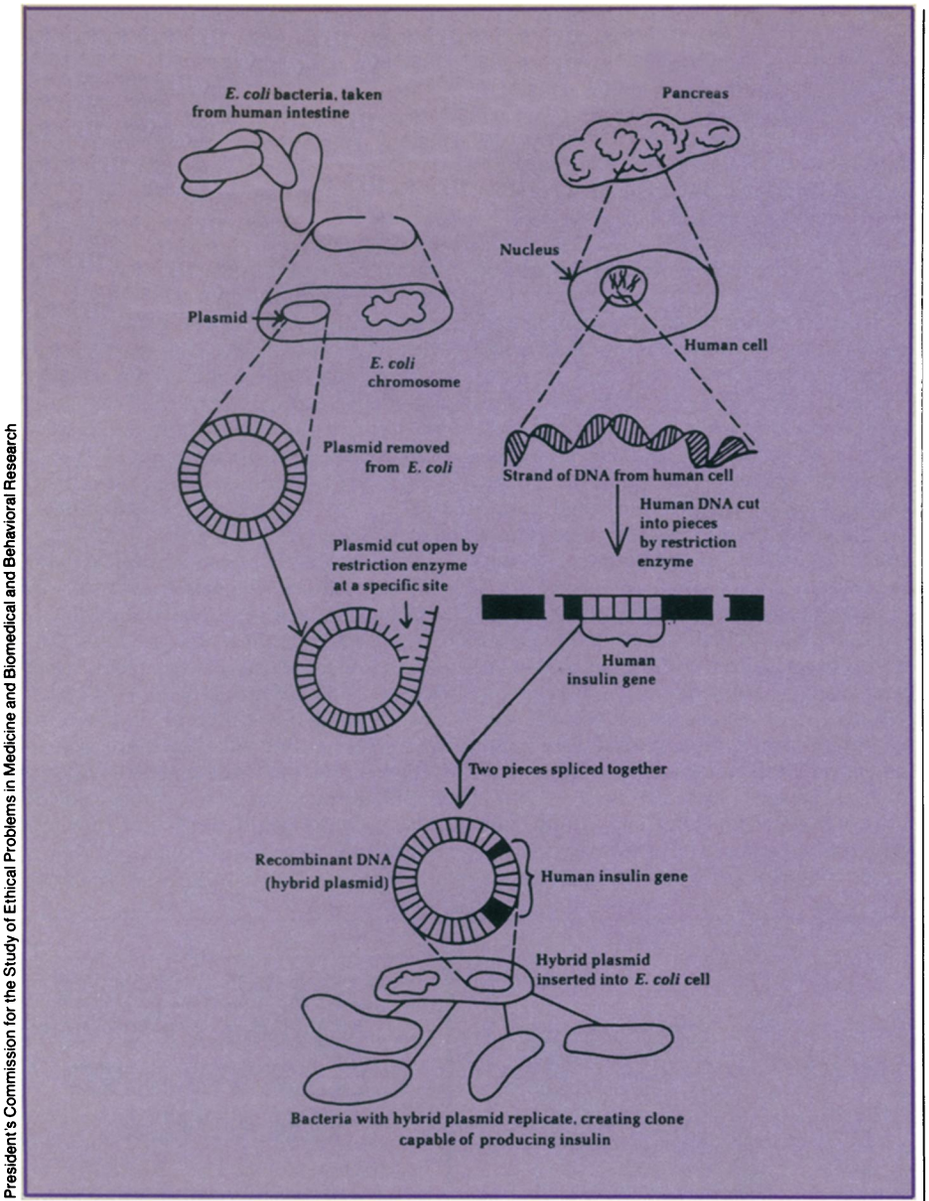
One of the earliest recombinant medical products to be approved for general use — human growth hormone — has long been known to be successful in treating children with inadequate supplies of their own growth hormone (SN: 10/26/85, p.263). This pure form of the hormone has replaced the pituitary human growth hormone derived from cadavers, which was used on children for a quarter of a century until 1985, when researchers recognized its tendency to transmit a rare, fatal viral disease (SN: 8/17/85, p.103).

By now, some 10,000 growth-hormone-deficient children worldwide have been treated successfully with pure, recombinant growth hormone and are growing at normal rates, reports Ron G. Rosenfeld, a pediatric endocrinologist at Stanford medical school. The next challenge, he says, is to find new uses for recombinant growth hormone and to make sure it is not overused.

One possible new use is in the treatment of osteoporosis, a bone-weakening disease that affects many elderly women, Rosenfeld says. Physicians have found that growth hormone secretion declines in elderly people, and particularly in postmenopausal women, and growth hormone is known to promote phosphate reabsorption, which helps strengthen bones, he says. However, he cautions, there is no complete research that supports this theory, and it remains "highly speculative."

Some physicians also have suggested using growth hormone to treat slow-healing bone fractures because it appears to stimulate cartilage and bone growth, Rosenfeld says. But this prospect, too, remains purely theoretical, he says.

Researchers have more solid reason to believe that recombinant growth hormone might help children who fail to grow normally even though they appear to have adequate supplies of their own natural growth hormone. Rosenfeld describes one 4-year-old girl with this condition, known as "normal variant short stature," who was the size of a 9-month-old baby but still walked, talked, jumped



Splicing human genes into bacteria enables the bacteria to manufacture human proteins.

and sang songs like a 4-year-old.

Physicians do not know what causes normal variant short stature, but one possibility is that children with this condition are unable to make use of their own growth hormone, Rosenfeld says. Early trials suggest that, for some as-yet-unknown reason, supplemental injections of recombinant growth hormone do foster growth in these children. But these studies have been in progress for only about a year, and it will take more time to determine the long-term effects, he says.

Recombinant growth hormone, like pituitary growth hormone before it, has attractive properties that make some people want to use it needlessly. And body builders, who know the hormone will promote muscle growth, are not the only ones inclined to abuse it, says Rosenfeld. Some parents ask for the hormone to make sure their normal children grow tall – in spite of the cost of treatment ($10,000 to $25,000 a year) and in spite of the risk of diabetes, one of the potential side effects of growth hormone.

"I've had families come to me and say, 'I don't care how much it costs. I don't care about side effects,' " he says. "We literally are turning away families with this attitude."

As medical researchers have gained more experience with recombinant medicines, they have learned to anticipate side effects, and not to expect miracles. No longer do researchers expect to clone "cures." Instead, they are learning – one by one – the complex ways in which individual human proteins behave and how to use these proteins to make modest, but significant, inroads against disease. □

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Annual Review of Computer Science, Vol.1 – Joseph F. Traub, Ed. The first volume in the new Annual Review series on computer science research. Annual Reviews, 1986, 459 p., illus., $39.

The Body – Anthony Smith. Originally published in 1968, this examination of the body, from skeleton to skin, has been revised and updated. The book is organized around human reproduction and development, discussing pregnancy, childbirth, heredity and the body's growth through puberty to maturity, old age and death. Organs and bodily systems are covered in this readable book on physiology and anatomy for the general reader. This revised edition was published in hardback in 1986 by Viking Press. Penguin, 1987, 548 p., paper, $7.95.

Cranium Crackers – Abbie Salny. A collection of 230 challenging mathematics, word and logic puzzles that are arranged from the least to the most difficult. The solutions are included at the end of the book. Dodd, 1986, 127 p., illus., paper, $6.95.

Human Culture: A Moment in Evolution – Theodosius Dobzhansky and Ernest Boesiger, edited and completed by Bruce Wallace. Examines the logic of evolution and the status of the human species with respect to evolutionary change. Presents a unique discussion of biological and cultural evolution and the various relationships between the two. Dobzhansky and Boesiger died in 1975 before completing the manuscript for this book. Wallace, a geneticist, completed and edited the book. Originally published in hardback in 1983. Columbia U Pr, 1986, 175 p., illus by Hans Erni, paper, $12.50.

A Leg to Stand on – Oliver Sacks. The author, a neurologist, tells of his experience as a patient after a leg injury sustained in a fall. Many themes are interwoven here, says Sacks in the preface, including the neuropsychological and existential phenomena associated with his injury and recovery, being a patient and returning to the outside world, the complexities of the doctor-patient relationship, a critique of current neurology and a vision of neurology of the future. Originally published in hardback in 1984 by Summit Books. Har-Row, 1987, 222 p., paper, $7.95.

The Man Who Mistook His Wife for a Hat: And Other Clinical Tales – Oliver Sacks. A collection of 24 case studies about seemingly untreatable patients with brain dysfunctions. In each of these readable case studies, Sacks looks for the person behind the sickness. He searches for the ways in which people compensate for loss, excess and aberration, and how the thread of identity can emerge unbroken despite severe handicaps. Originally published in hardback in 1985 by Summit Books. Har-Row, 1987, 243 p., illus., paper, $7.95.

The Sea Turtle: So Excellent a Fishe – Archie Carr. First published in 1967, this account of sea turtles by one of the world's experts on marine turtles has been updated with an epilogue that highlights recent developments in this field. New photographs and maps also have been added to this revised edition. The revised edition was published in hardback by Scribner in 1984 as *So Excellent a Fishe.* U of Texas Pr, 1986, 280 p., illus., paper, $9.95.