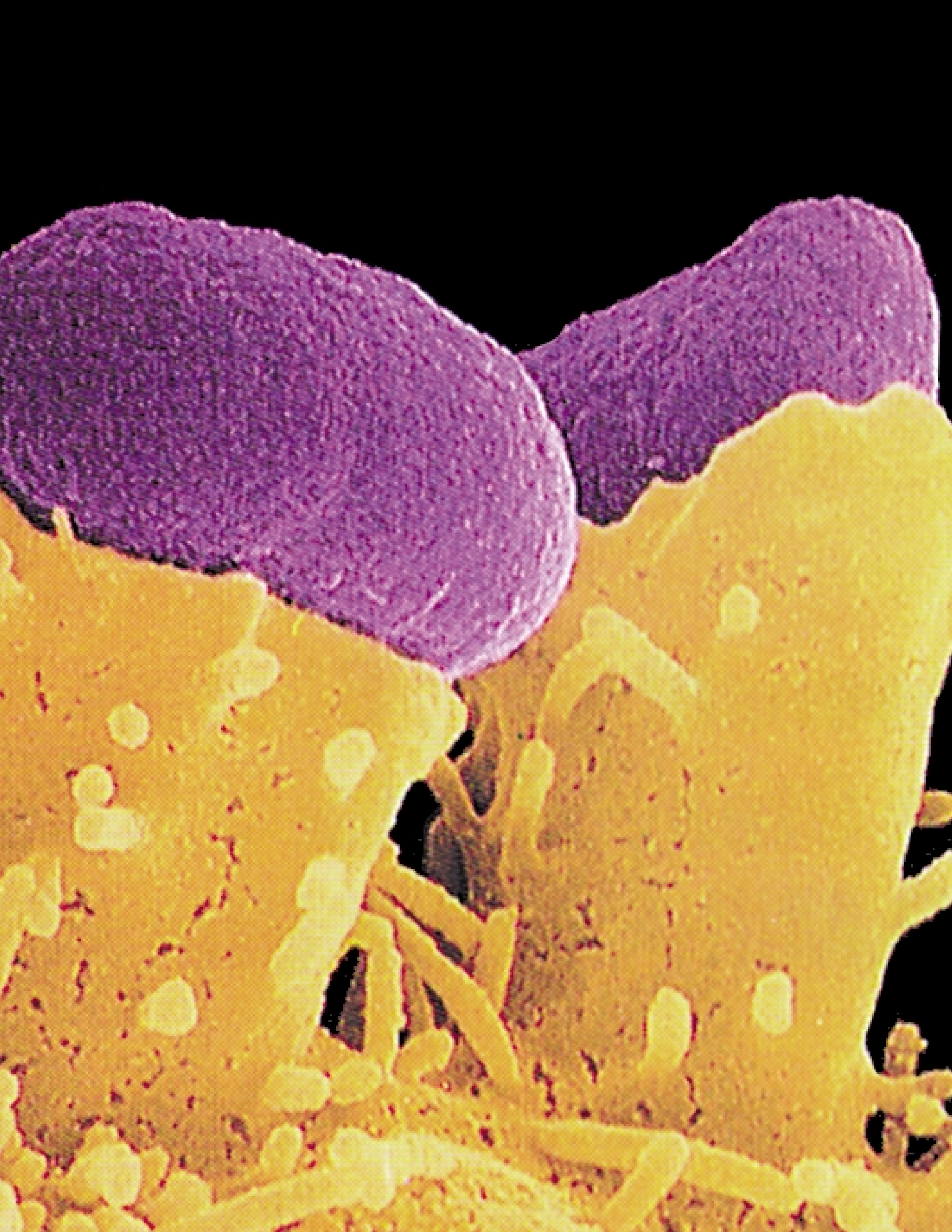


Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. They include disorders of the gastrointestinal tract, liver, gallbladder, and pancreas. Nutrition research is important to understanding, treating and preventing many diseases such as type 2 diabetes, obesity, chronic renal disease, heart disease and cancer.

Understanding the Many Facets of Obesity

Obesity is the most common and fastest growing health problem in the U.S. Individuals who are overweight or obese are at heightened risk for developing a number of diseases, including type 2 diabetes, heart disease, stroke, and some forms of cancer. Reportedly, obesity is also the second most common cause of preventable death in the U.S. Hence, questions arise regarding the effects of diet and lifestyle changes on quality of life and disease outcomes. Several ways of measuring overweight or obesity exist, including Body Mass Index, or BMI (see the following sidebar on different measures). BMI is a ratio derived from a person's weight and height; people with a BMI of 25-30 are considered overweight, while those with a BMI higher than 30 are classified as obese. Based on BMI, more than half of adults in the U.S. are overweight, and nearly one quarter are obese.

The dramatic impact of obesity on the health of Americans was vividly illustrated in a recent study of coronary heart disease from 1980 to 1994 among women participating in the Nurses' Health Study. A large sample population, high rate of follow-up, and exacting detail regarding dietary and other lifestyle factors offered a unique opportunity to examine trends over time in the incidence of coronary disease and the extent to which diet and lifestyle might account for these trends. Researchers observed a significant decline in the incidence of coronary disease over a twelve year period among study participants. Reduction in smoking, improvement in diet, and an increase in the use of hormone replacement therapy explain much of this decline. However, obesity increased significantly in this cohort. Because obesity is a strong risk factor for type 2 diabetes and cardiovascular disease, the increase in its prevalence appears to have prevented what would have been an even greater decline from occurring in the incidence of coronary disease. These findings emphasize the importance of diet and lifestyle in the primary prevention of coronary disease.

Obesity is a consequence of greater energy intake in the form of calories than of energy expenditure through metabolic processes and physical activity. One possible therapeutic approach to combating weight problems is to control the number of fat cells developed by the body. The regulation of cell growth in developmental processes is orchestrated by such molecular events as the expression of signaling proteins known as "Wnts." Pre-adipocytes are precursors to mature fat cells. Recent studies using cellular models tested whether *Wnt* expression in pre-adipocytes affected their ability to differentiate into mature cells. When researchers repressed other proteins in the signaling pathway critical for fat cell development, the pre-adipocytes failed to mature. Strategies that aim at enhancing or mimicking these signals may become an effective treatment for obesity.

Control mechanisms in the central nervous system can also regulate food intake, fat storage and body weight. Studies of various hormones, including molecules produced by the brain and those produced in other

While some forms of the bacterium *E. coli* are harmless and even beneficial, other forms are responsible for foodborne illnesses, including some that can be fatal. This photograph shows two disease-causing *E. coli* bacteria (purple) infecting a gut cell (orange). After the bacteria attach to the host cell, they cause it to form a pedestal-like structure. Photo: Reprinted with permission, from Rosenshine I, Ruschkowski S, Stein M, Reinscheid DJ, Mills SD, and Finlay BB. *EMBO J* 15(11):2613-2624, 1996.

tissues, have shown that they can significantly influence feeding behavior and energy expenditure. For example, mice engineered to lack insulin receptors only in their brains eat more and exhibit elevated levels of body fat in contrast to normal mice. This finding indicates that insulin acts on the brain and plays a role in influencing food intake in addition to its better-characterized roles in glucose metabolism. Hormones produced in the brain are also believed to be involved in regulating food intake.

The melanocortins are hormones produced in a region of the brain known as the hypothalamus. Mice whose melanocortin signaling pathway has been disrupted display a variety of defects in feeding and fat storage. Unlike normal mice, those lacking the melanocortin-4 receptor gene (*MC4-R)* exhibit increased food intake and become obese. Mice lacking the related *MC3-R* gene were found to possess 50-60 percent more body fat than normal mice, although this increase in body fat did not result in the mice being overweight. These and other data suggest that the primary role of *MC4-R* may be to control appetite while *MC3-R* influences how energy from food is used and stored.

Studies of fat metabolism are another route to new approaches for treating obesity. Scientists have now identified several hormones produced in fat cells, which target the brain's hypothalamus to regulate body weight, either by stimulating or suppressing appetite. Two recent studies have explored the pathways of triglyceride metabolism as a means of controlling obesity.

Triglycerides are composed of glycerol and fatty acids and provide the body with a source of stored energy for metabolic functions. The last step in the synthesis of triglycerides is mediated by an enzyme called "Dgat." Because triglycerides play such an important role in metabolic functions, this enzyme was initially believed to be required for survival. Researchers have now bred a strain of genetically engineered mice that no longer has the gene that codes for this enzyme. Surprisingly, these mice grow and develop normally and are even capable of synthesizing triglycerides. These findings suggest that there are alternative pathways for triglyceride synthesis that are not dependent on Dgat. Importantly, these mice are lean and resistant to diet-induced obesity. The leanness is the result of an increased metabolic rate and higher levels of physical activity, which result in increased energy expenditure without a compensatory increase in appetite and food intake. Because these mice have appropriately low serum levels of leptin, an important hormone in weight regulation, they demonstrate that Dgat does not act through a leptin metabolic pathway.

In related work on fat metabolism, investigators tested the effect of treating mice with two fatty acid synthetase (FAS) inhibitors, cerulenin and the synthetic compound, C75. Results demonstrated a profound dose-dependent weight loss with the administration of either FAS inhibitor. Additional studies revealed that food consumption was significantly reduced over the first 24 hours after treatment with C75-mediated FAS inhibitor, then returned to normal during the following 24 to 48 hours. The treated mice lost both lean body mass, as well as adipose mass, which is the same pattern of weight loss in fasting. However, treated mice lost 40 percent more weight than paired, equally-fed mice. It is known that C75 inhibits neuropeptide Y (NPY), a protein that regulates feeding status and adiposity, providing evidence that decreased appetite, at least in part, results from blocking NPY-induced feeding. Researchers found that C75-mediated FAS inhibition results in increased levels of the FAS substrate, malonyl-CoA, and that an inhibitor of malonyl-CoA restores appetite. These results support the theory that malonyl-CoA mediates the metabolic signal for appetite inhibition.

Finding ways to enhance the body's burning of calories, called thermogenesis, is yet another therapeutic approach to obesity. Under normal conditions, the breakdown of fat is coupled to the production of chemical energy for use by the cells of the body. Because of this, people who want to lose weight are advised to modify their diets, in order to decrease the amount of food energy they consume, and to exercise, in order to increase the amount of energy they expend. When this happens, the body uses stored fat as an energy source. However, there may be other ways to achieve the dissipation of stored fat. In some fat cells, the presence of "uncoupling proteins" severs the link between fat metabolism and chemical energy production, and the energy that usually drives a series of chemical reactions is instead dissipated as heat. To investigate whether this uncoupling phenomenon in other tissues might alter metabolism, scientists engineered mice that possess an uncoupling protein in their skeletal muscle, a major site of energy metabolism. These mice exhibit elevated rates of metabolism in both resting and active states. Compared to normal mice, "uncoupled" mice are leaner when main-

Who Should Lose Weight?

Am I overweight or obese? At first, it seems like an easy question to answer. However, defining overweight and obesity proves more difficult than might be expected. At what point do the extra pounds cease to be an annoyance and become a serious threat to health? As Americans become heavier and heavier, the toll of obesity-related diseases such as diabetes and cardiovascular disease becomes greater. To appreciate the impact of excess weight on disease, one must realize that overweight and obesity are conditions that are defined by more than just total body weight as shown on a bathroom scale. Because of this, several methods to measure body mass and body fat have been developed.

Body Mass Index: Among health care professionals, perhaps the best known method for assessing body size is the body mass index, or BMI. BMI is a value derived from a person's height divided by his weight. Specifically, weight in kilograms is divided by height in meters, squared. Persons with a BMI of between 25 and 30 are considered to be overweight, while those with a BMI greater than 30 are classified as obese. For example, a person who is six feet tall and weighs 175 pounds has a BMI of 23.7, a value that is within normal range. If a person of the same height weighed 200 pounds, his BMI would rise to 27.1, indicating overweight. At 230 pounds, his BMI would be 31.2, indicating obesity. BMI represents a valuable and easy-to-calculate manner of determining whether a person is obese, and BMI may be used by both men and women to estimate their relative risk of developing disease.

$$\text{BMI} = \left[\frac{\textit{Weight(kg)}}{\textit{Height(m)} \times \textit{Height(m)}} \right]$$

Waist Circumference: Although BMI is a widely used and valuable tool, it is not perfect. Individuals whose weight is predominantly muscular, as well as pregnant women, may have elevated BMI values even though they are relatively healthy. Because of these and other limitations of BMI, scientists and physicians have looked for alternative ways to assess body fat in order to determine the likelihood of disease development. Studies have shown that people whose fat is primarily localized in their abdomens—with so-called "apple" shape—are at greater risk of developing complications, in particular cardiovascular disease, than individuals of the same weight whose fat is distributed in their hips and thighs—with so-called "pear" shape. These differences in the distribution of fat have led to another method for identifying individuals at risk—using a simple tape measure to determine waist circumference. In men, a waist circumference of 40 inches or greater places individuals at risk of developing a number of obesity-related diseases; in women, a waist circumference of greater than 35 inches is considered unhealthy. Importantly, many men store their fat in their abdominal region, in contrast to many women, whose fat is more likely to be deposited in the thighs and gluteal region. Although women tend to have more body fat than men, the fact that men are more likely to store it abdominally means that the fat in men may pose a greater health risk than that in women.

Comparative Measurements: The waist-to-hip ratio, a comparison of waist and hip circumferences, provides important information not only about the amount of fat a person carries but the proportion of abdominal fat and, by extension, relative risk of cardiovascular complications. People with a higher ratio are at increased risk of developing diseases associated with overweight. This measurement is informative because it provides a somewhat more refined measure of overall fat distribution. In general, men with a waist-to-hip ratio of greater than 1.0 and women with a ratio greater than 0.8 are considered to have an excess accumulation of fat in their abdomens. For example, a woman with a waist measurement of 30 inches and a hip measurement of 40 inches would have a waist-to-hip ratio of 0.75. In a recent study, women with a ratio greater than 0.76 had twice the risk of developing coronary disease than those whose ratio was 0.75 or lower.

Other Ways To Measure Body Fat: Another way to measure body fat is to look at subcutaneous fat—the fat

Who Should Lose Weight?

beneath the skin. This measurement is obtained using calipers, pincher-like devices that determine the thickness of the subcutaneous fat layer. The Standardized Skinfold measurement involves measuring the thickness of several defined folds of skin sampled at fixed points along the body. Based on a mathematical formula, the thickness of these folds is used to compute a person's approximate body fat.

Conclusions: Although methods of measuring overweight and obesity may vary, it is clear that excessive weight poses a serious risk to health. While the cut-off points in each measurement may seem arbitrary, they represent an effort to quantify an essentially imprecise variable. Each method for determining body fat has advantages and disadvantages, and no single value should be examined without considering the overall health of the individual. However defined, overweight and obesity contribute to the development of a number of debilitating diseases, including arthritis, heart disease, and diabetes. For example, the increasing prevalence of weight problems among young people is thought to be a driving force behind the alarming rise of type 2 diabetes in children. It is entirely possible that, if untreated, such individuals could face many years fending off in mid-life the serious complications of diabetes, including blindness, amputation, and kidney failure. It is therefore of vital importance that the problems of overweight and obesity be addressed aggressively by researchers, physicians, and patients.

tained on a regular diet and gain less weight when placed on a high fat diet. This research suggests that the dissipation of fat energy as heat through the "uncoupling" process might represent a viable strategy for preventing or treating obesity.

Clarifying the mechanisms responsible for the normal regulation of food intake and body weight provides insight into how the system may be altered in diseases characterized by abnormal regulation of energy balance, such as obesity. There is an increasing recognition that a complex interplay exists between genetic factors, the environment, and a host of biological factors. New mouse models of obesity are spurring advances on several fronts: fat cell development; controls on the efficiency of fat absorption and food intake; and variations in the rate and manner in which fat is stored and metabolized. Studies of fat metabolism are yielding useful clues about how energy regulation will help pave the way to future interventions to mitigate or prevent obesity. Knockout mice lacking the enzyme Dgat provide an important tool for investigating triglyceride synthesis and its relationship to obesity. Studies using this tool suggest that molecules that target triglyceride synthesis may prove to be an exciting new approach to treatment. At the same time, drugs that mimic the actions of C75 by inhibiting FAS synthetase may cause appetite inhibition, while sustaining an increase in metabolic rate, and would possibly provide ideal agents for treatment of obesity. New ways of inducing the body to burn fat are being explored. Identifying obesity genes in both animal and human models will continue to enhance understanding of the basis for weight control at both the molecular and clinical levels and lead to future targeted treatments for obesity. While identifying obesity genes is critical, programs that will successfully encourage increased physical activity and healthful eating remain of paramount importance for the prevention of obesity.

The NIDDK has a strong program of research on and related to obesity—both as a serious risk factor for type 2 diabetes and as an independent health problem. The Institute established a National Task Force in the Prevention and Treatment of Obesity, which provides science-based guidance to aid research strategies and to generate public health messages. The NIDDK also supports Obesity/Nutrition Research Centers and Clinical Nutrition Research Units. A multi-center clinical trial will examine the health effects of voluntary weight loss in obese diabetes patients. The trial is called "Look AHEAD," Action for Health in Diabetes. The NIDDK's public education efforts related to obesity include the Weight Control Information Network, and the National Diabetes Education Program, a cooperative initiative with the Centers for Disease Control and Prevention and approximately 200 public and private partnership organizations. Underlying all these programs is a solid base of fundamental research on biologic processes such as nutrient metabolism and how it is influenced by genetic and environmental factors.

Brüning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Müller-Wieland D, and Kahn CR. Role of brain insulin receptor in control of body weight and reproduction. *Science* 289(5487):2122-2125, 2000.

Butler AA, Kesterson RA, Khong K, Cullen MJ, Pelleymounter MA, Dekoning J, Baetscher M, and Cone RD. A unique metabolic syndrome causes obesity in the melanocortin-3 receptor deficient mouse. *Endocrinology* 141(9):3518-3521, 2000.

Hu FB, Stampfer MJ, Manson JE, Grodstein F, Colditz GA, Speizer FE, and Willett WC. Trends in the incidence of coronary heart disease and changes in diet and lifestyle in women. *N Engl J Med* 343(8):530-537, 2000.

Li B, Nolte LA, Ju JS, Ho Han OD, Coleman T, Holloszy JO, and Semenkovich CF. Skeletal muscle respiratory uncoupling prevents diet-induced obesity and insulin resistance in mice. *Nat Med* 6(10):1115-1120, 2000.

Loftus TM, Jaworsky DE, Frehywot GL, Townsend CA, Ronnett GV, Lane MD, and Kuhajda FP. Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* 288(5475):2379-2381, 2000.

Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, and MacDougald OA. Inhibition of adipogenesis by *Wnt* signaling. *Science* 289(5481):950-953, 2000.

Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, Sanan DA, Raber J, Eckel RH, and Farese RV Jr. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking *Dgat*. *Nat Genet* 25(1):87-90, 2000.

Understanding the Causes of Fatty Liver

A new focus of digestive diseases research is a common liver disease of unclear origin marked by the accumulation of fat in the liver (steatosis) and inflammation and cell death (hepatitis). This complex of symptoms is known as non-alcoholic steatohepatitis (NASH). The condition resembles alcoholic liver disease, but occurs in patients who have no significant history of alcohol consumption. The true incidence and prevalence of NASH are not known. Typically occurring in middle-aged persons who are overweight and diabetic, it can also occur in individuals of normal weight, in non-diabetics, in children, and in the elderly. Usually asymptomatic, NASH is often discovered when an individual is found to have elevated serum liver enzyme levels. The disease can progress to cirrhosis and end-stage liver disease, and possibly accounts for 10 to15 percent of liver transplants in the U.S. The association of steatohepatitis and liver fibrosis with obesity and type 2 diabetes can be explained by elevated serum insulin and the insulin-resistant state found in these conditions.

Fatty liver has no known treatment; however, its association with high levels of insulin and insulin-resistance prompted researchers to assess the possibility that insulin-sensitizing agents might be beneficial. Hence, a recent study tested the efficacy of the diabetes medication metformin as a treatment for fatty liver disease in obese mice. These mice develop high insulin levels, insulin-resistance, and fatty livers because of an inherited deficiency of the appetite-suppressing hormone, leptin. The metformin therapy eliminated fatty liver disease in this mouse model, leading to investigation of potential mechanisms for this effect. Subsequent studies revealed that metformin inhibits expression of liver tumor necrosis factor-alpha, an immune regulatory substance known to inhibit the dispersal of insulin receptor-initiated signals in many cells, including liver cells. The researchers also showed that metformin reversed several responses induced by this factor, which are likely to promote fatty liver and cell death. These findings suggest a mechanism of action of metformin, and also point the way to novel therapeutic targets in obesity-related insulin-resistance.

Future efforts will be directed toward expanding research on the origin and development of NASH to clarify the cellular, hormonal and genetic mechanisms by which injury occurs in this disease. Particular focus will be placed on the generation of new animal and cell culture models. Plans are under way for design and implementation of a database and clinical research network for the purpose of studying the natural history, complications, contributing factors and therapy of NASH. This initiative will include clinical centers and a data coordinating center to enroll a large cohort of patients. These patients would be followed in a natural history study and undergo clinical investigation to develop criteria for diagnosis and identification of stages of the disease. The database would be used for epidemiological studies of risk factors, and for providing a group of well-characterized patients who may wish to participate in future clinical investigation.

Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, and Diehl AM. Metformin reverses fatty liver disease in obese, leptin-deficient mice. *Nat Med* 6(9):998-1003, 2000.

Resolving Questions about Hepatitis C

One of the most serious attacks on the liver is from hepatitis viruses. The NIH has made substantial progress in combating hepatitis, through the efforts of several institutes, including the NIDDK and the National Institute of Allergy and Infectious Diseases, as well as the Warren Grant Magnuson Clinical Center. The most recent acknowledgement of NIH contributions to this field of research was the 2000 Lasker Award for Clinical Medical Research given to Dr. Harvey Alter of the NIH Clinical Center and Dr. Michael Houghton of Chiron Corporation. The citation reads: "For pioneering work leading to the discovery of the virus that causes hepatitis C and the development of screening methods that reduced the risk of blood transfusion-associated hepatitis in the U.S. from 30 percent in 1970 to virtually zero in 2000."

Infection with the hepatitis C virus (HCV) affects approximately four million Americans and is believed to be the most common cause of chronic liver disease, cirrhosis, and liver cancer in the Western world. It is often asymptomatic, but in 20 to 30 percent of patients, chronic hepatitis C infection advances to progressive liver disease and, ultimately, to cirrhosis and liver failure within ten to thirty years. In the 1970s, receiving a blood transfusion carried a high risk of hepatitis infection. It was for this reason that Dr. Harvey Alter and colleagues began clinical research studies of transfusion-associated hepatitis. An enzyme called alanine aminotransferase leaks from the liver into the bloodstream when the liver is infected. By measuring the levels of this enzyme, researchers were able to determine if donor or recipient blood were infected. From the results of these studies, the researchers found that recipients of transfusions had a one-in-three chance of becoming infected with hepatitis. Most of the contaminated blood came from paid donors who had hepatitis B. These and other epidemiological studies resulted in an all-volunteer donation system and antibody testing of all donor blood, which reduced the incidence of hepatitis by 50 percent.

Screening individuals with transfusion-associated hepatitis for the then-known causes of hepatitis, hepatitis A virus and hepatitis B virus, led researchers to conclude that there was yet another form of hepatitis, which they called "nonA, nonB hepatitis" (NANBH). After years of painstaking research, a causal agent was described for this third form of hepatitis, which was named hepatitis C (HCV). Subsequently, HCV was shown to be the cause of over 90 percent of cases of NANBH. Sensitive diagnostic antibody tests were developed that permitted blood to be screened for hepatitis C. As a result, post-transfusion hepatitis C has been virtually eliminated. With the establishment of public health measures, the incidence of new cases of hepatitis C has dramatically declined in the U.S., although chronic cases are being uncovered in large numbers due to the long incubation period of the virus.

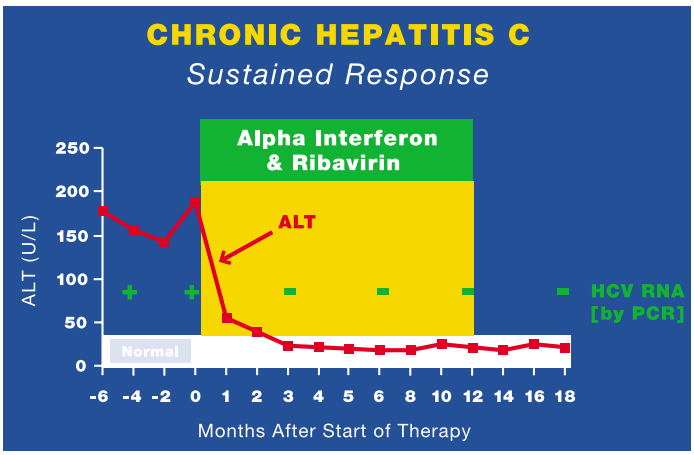
The first successful antiviral therapy with alpha interferon was reported by an NIDDK scientist in 1986. Administered by injection for six to twelve months, this therapy led to improvement of the disease in at least half of the HCV patients treated, and to sustained improvement in 15 to 25 percent of patients after treatment ceased. Continuing to improve the rate of patient response to this therapy has been a major research challenge. The aim of a recent multicenter clinical trial was to compare the efficacy and safety of alpha interferon alone, or combined with the antiviral agent ribavirin, for the initial treatment of chronic hepatitis C. Results showed that sustained absence of virus and general improvement were greater in patients receiving combination therapy *versus* alpha interferon alone, and the combination therapy was determined to be safe. A significant research advance is the ability to enhance the response during treatment, and subsequently, to prevent relapse in chronic hepatitis C patients. Yet, at least three critical questions need to be resolved in order to move treatment for hepatitis C forward.

First, why is it that some patients recover from acute hepatitis C virus infection while others have chronic infection? Some clues have been gleaned from a recent study in which researchers examined serum samples from patients infected with the same strain of HCV during an outbreak that occurred in the late 1970s. Long-term follow up, as well as samples of serum and T cells (cells of the immune system used by the body in its fight against infection), were available for a large number of patients 10 and 20 years after onset of the disease. The investigators found that persons who had developed a chronic infection had high levels of antibodies against HCV (B cell, humoral immunity) but had poor HCV-specific T cell responses (cellular immunity). In contrast, patients who had acute hepatitis C in the 1970s, but then recovered, continued to have strong T cell responses to HCV 20 years later, but had low levels of antibody. Although all

had tested antibody-positive 10 years after onset, 42 percent of those who recovered had no detectable antibody to HCV after 20 years. The results of this study indicate that T cell responses to HCV are long-lived and are perhaps a better biomarker for identifying patients with prior HCV infection and recovery than the number of HCV antibodies detected. Furthermore, T cell responses appear to be key for clearance of virus and recovery from infection. Based on these findings, it is possible that vaccines against HCV may need to induce vigorous and long-lived T cell immunity rather than B cell immunity.

A second central question still at issue is whether responses to therapy can be sustained and can result in permanent remissions or cure of the disease. To define the long-term survival and clinical outcome of treatment with alpha interferon for patients with chronic hepatitis C, a recent study evaluated the clinical, histological, and virological outcomes of ten patients treated between 1984 and 1987 with alpha interferon. Prior to therapy, all ten had evidence of hepatitis C virus, elevations of liver enzymes, and chronic hepatitis with fibrosis on liver biopsy. At the time of the final follow-up, which averaged ten years from initiation of therapy, the patients with sustained virological responses had no symptoms or physical findings of liver disease—hence, a resolution of their disease. These results demonstrated that patients who have no evidence of the virus for six months after cessation of therapy have a favorable long-term clinical and histological outcome. Increased response rates and sustained improvement place research at the threshold of a cure for hepatitis C viral infection.

A third question is what are the reasons for the disproportionately heavy burden of hepatitis C on African Americans and what can be done to solve this problem? The results of several studies have revealed that the prevalence of hepatitis C among African Americans is higher than among Caucasians, and their response rate to treatment with interferon, with or without ribavirin, for chronic HCV infection is lower than for other groups. African Americans with HCV also have a significantly higher incidence of liver cancer. Of the known genetic subtypes of HCV, one has been shown to have the greatest resistance to treatment. This genetic subtype is highly prevalent in African Americans and is believed to contribute to their lower response to antiviral therapies. In order to address these disparities, NIDDK held a workshop on "Hepatitis C in African Americans,"



Levels of the liver enzyme known as alanine aminotransferase (ALT) are elevated in the blood of people with hepatitis C (HCV) and are indicative of active liver damage. In the graph shown above, a person with hepatitis C who had levels of ALT (red line) about four times higher than the range considered normal (light blue bar), was treated for twelve months with a combination of alpha interferon, a cytokine, and ribavirin, an antiviral agent (duration of treatment indicated by the yellow box). Within three months, ALT levels were within the normal range. The presence of HCV genetic material, which was readily seen prior to the onset of therapy, could not be detected once treatment was started, even using an ultra-sensitive assay known as PCR (green plus and minus signs). Importantly, ALT levels and HCV remained low, and HCV was undetectable even after the discontinuation of treatment after twelve months. Graph: Dr. Jay Hoofnagle, NIDDK.

which focused on exploring factors that could be responsible for their different outcomes. The NIDDK is initiating a multicenter clinical trial to study viral resistance to interferon therapies, with a specific focus on African Americans.

Currently, therapy for hepatitis C is recommended only for patients with chronic infection and raised serum levels of the enzyme aminotransferase. The recommended regimen of combination therapy carries with it the potential for serious side effects and is also very costly. However, little information exists on the efficacy and relative safety of this conventional therapeutic guidance. Beginning treatment early after exposure could prove to be more effective in eradicating infection than starting treatment once chronic hepatitis develops. To resolve this issue, the NIDDK is supporting a collaborative randomized trial to determine the most effective time to begin treatment. Thirty clinical centers across the U.S. are expected to participate in the trial, which will treat and monitor health care professionals who have contracted the disease through an accidental stick with a needle used to treat an individual with HCV.

While research on the cause and progression of HCV continues at a brisk pace, the ultimate goal of the NIDDK is to develop a vaccine to prevent the disease. However, several factors have impeded progress: (1) only low levels of viral particles are found in infected individuals; (2) researchers have been unable to grow the virus in cultured cells; and (3) there has not been a convenient animal model. An encouraging step forward is that NIDDK scientists have now developed a model for producing and purifying HCV-like particles in insect cells. These noninfectious particles lack the genes required for viral replication, thereby providing an excellent candidate for a vaccine. Recent experiments analyzing the structural features and antigenic composition of these particles indicate that they are capable of inducing an HCV-specific immune response, findings that support the promise of an HCV vaccine.

Significant progress has been made over the past 30 years in the fight against hepatitis C. However, much more research needs to be done to answer outstanding questions and to treat this disease effectively in all populations. Thus, the NIDDK is continuing its strong support of research and clinical trials designed to overcome this often fatal disease. This work includes studies of the natural history and epidemiology of hepatitis C, and research directed at developing a vaccine.

Takaki A, Wiese M, Maertens G, Depla E, Seifert U, Liebetrau A, Miller JL, Manns MP, and Rehermann B. Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. *Nat Med* 6(5):578-582, 2000.

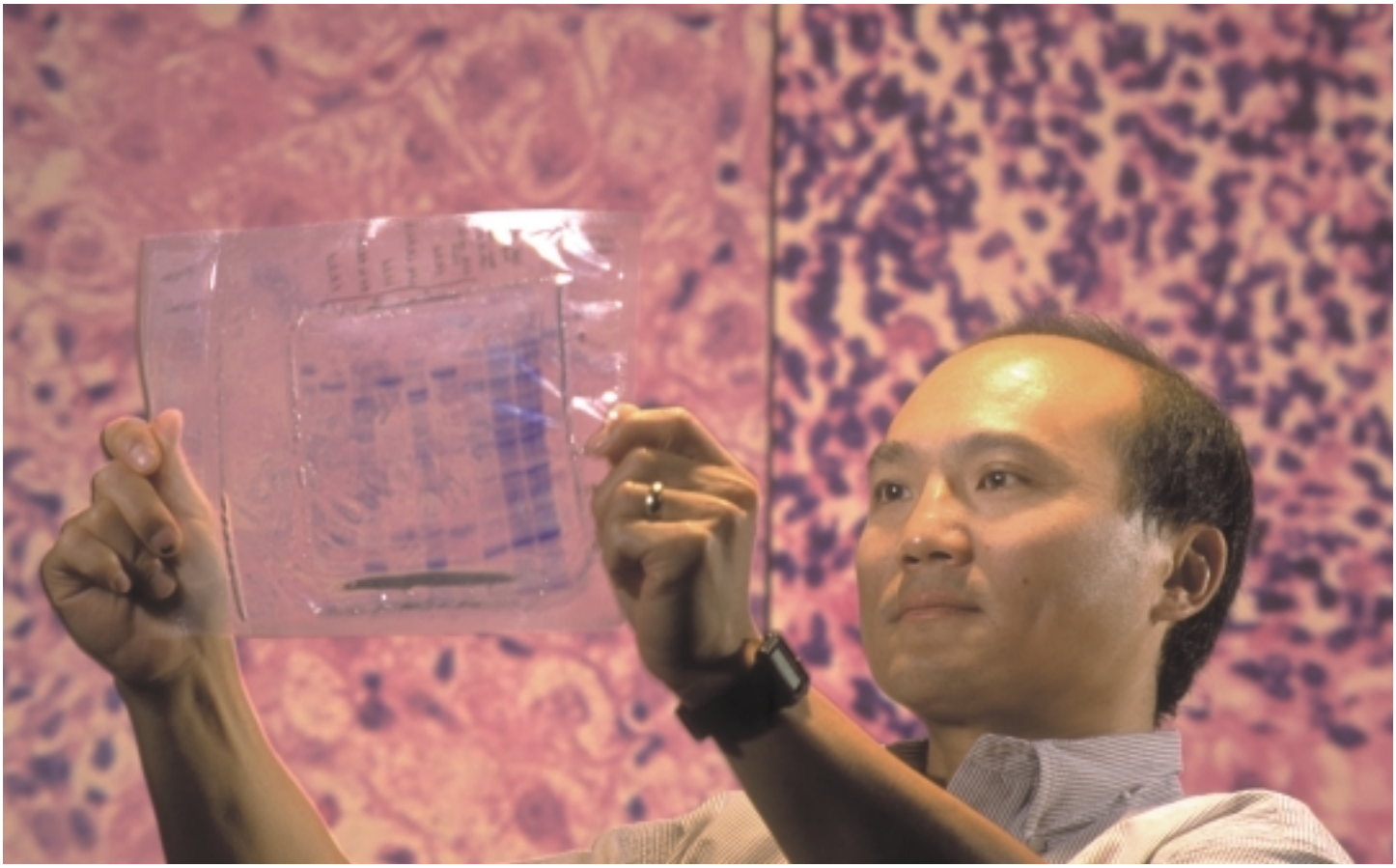


Photo: Mr. Richard Nowitz.

PATIENT PROFILE

Judie Keithley – Hepatitis C

Judie Keithley, a teacher of children with learning disabilities, thinks she contracted the hepatitis C virus (HCV) as the result of a blood transfusion she received as a young child. In Judie's case, the virus, one of the most prevalent causes of chronic liver disease in the U.S., stayed dormant for decades, not causing symptoms or any signs of liver damage. It wasn't until she was tested and treated in 1991 for a gastrointestinal virus contracted on a family camping trip that Judie learned, completely by accident, that she was infected with HCV.

Shortly after she was diagnosed with HCV, a routine test also showed that Judie had a vascular condition known as cryoglobulinemia. This rare immune system disease was a direct result of her HCV infection. Along with HCV infection, this "secondary disease" started causing Judie joint pain, energy loss, skin rashes, and severe depression. This complex of symptoms has made Judie's disease especially difficult to manage. The only means of treating her cryoglobulinemia was by treating her hepatitis C infection.

Today, Judie, 57, is still infected with HCV. However, as a result of receiving the most advanced treatments available for hepatitis C, Judie is able to live a fairly normal life. Her goal is to stay as healthy as possible, until an effective treatment or cure for hepatitis C is found.

About Hepatitis C

Hepatitis C is a blood-borne disease that causes inflammation and damage to the liver. If hepatitis C is advanced, the liver can no longer perform its life-supporting tasks of controlling metabolism, storing energy, making blood proteins and clotting factors, removing drugs, and breaking-down products from the blood. The liver is the metabolic factory of the body that gives one health, energy, and stamina. When the liver is inflamed and injured, a person can feel tired, have an increased need for sleep, and less energy to get through a routine day of activity.

Judie Keithley was diagnosed with hepatitis C in 1991. Judie's disease has been particularly unresponsive to treatment regimens that are being tested in research studies. After several attempts at treatment, her most recent round of therapy has left her feeling better. "I feel as if I have my life back," she says.

Infection with the hepatitis C virus affects approximately four million Americans and ranks second only to alcoholism as the most common cause of chronic liver disease, cirrhosis, and liver cancer in the Western world. It is often asymptomatic, but in 20 to 30 percent of patients, chronic hepatitis C infection advances to progressive liver disease, and ultimately to cirrhosis and end-stage liver disease within 10 to 30 years.

HCV was not discovered until 1989. Since then, there has been real progress in combating the disease—including use of the drug alpha interferon, as well as the combination of interferon with another antiviral drug, called ribavirin. What is most needed, however, is a vaccine to prevent hepatitis C; but, finding a vaccine has remained an elusive goal. The major problem in research into a vaccine is that the hepatitis C virus does not grow outside of the human body.

A serious challenge in management and treatment of chronic hepatitis C is that the disease varies greatly in its course and outcome, from being mild and asymptomatic in some patients to being severe and resulting rapidly in cirrhosis and in liver cancer in others. One of the most unusual features of hepatitis C is the specific condition that Judie has, her "secondary disease," known as cryoglobulinemia. In this condition, the virus combines with antibody in the blood and causes injury to blood vessels in the skin, joints,

lungs, kidneys, and nerves. Judie's case was relatively severe. In addition to high liver enzymes, she says: "I had lots of joint pain and swelling in my legs and feet and occasionally could barely walk." Shortly after her diagnosis, Judie joined a hepatitis C support group to learn as much as she could about the disease. She heard about interferon, the only treatment for hepatitis C at the time. "People in the support group kept telling me how disorienting the side effects of interferon can be," she said. As her condition progressively worsened, her physician recommended her to the NIDDK Division of Intramural Research, for participation in a research study. With trepidation, Judie went to the National Institutes of Health Clinical Center and enrolled in the research protocol.

Before 1992, there was no effective diagnostic test for hepatitis C. Some people received infected blood through blood transfusions or organ transplants. Today, hepatitis C is spread by:

- **Sharing drug needles.**
- **Getting pricked with a needle that has infected blood on it.**
- **Having sex with an infected partner (although infection through this route is not frequent).**

Treating the Disease

As part of NIDDK's studies on hepatitis C, Judie was treated with interferon injections for six months. The drug dramatically reduced her liver enzymes and relieved some of her rashes and joint pain. It also lowered her level of the hepatitis C virus but did not rid her of it. When the interferon was stopped, the virus levels and all of Judie's original symptoms returned. This was discouraging because for the six months that she was on treatment, "the interferon itself made me feel rotten," she says.

The return of the cryoglobulinemia and joint pains led Judie to accept enrollment in another research study, this time of long-term use of interferon for her hepatitis C. Although the drug helped control the virus and her cryoglobulinemia symptoms, the side effects of interferon wore on her more and more. "I got through it because I knew it was the interferon causing these side effects, and not me."

After being on interferon for four years, Judie was offered treatment with another research approach—the

HEPATITIS C FACTS

- **The hepatitis C virus (HCV) is a blood-borne virus and one of the most prevalent causes of chronic liver disease in the U.S.**
- **Almost four million Americans have antibody to HCV, indicating ongoing or previous infection with the virus.**
- **Hepatitis C causes an estimated 8,000 to 10,000 deaths annually in the U.S.**
- **Chronic hepatitis C varies greatly in its course and outcome, from patients who have no signs or symptoms of liver disease, to those who develop liver cancer.**
- **The disease disproportionately affects minority populations, and African Americans respond more poorly to treatment than do other groups.**
- **Currently, there is no vaccine for hepatitis C.**
- **The only means of preventing new cases of hepatitis C are to: (1) screen the blood supply; (2) encourage health professionals to take precautions when handling blood and body fluids; and (3) inform the public about high-risk behaviors.**

addition of the antiviral drug ribavirin to interferon treatment. A large, multicenter and multinational study had just shown that combining ribavirin with interferon was more effective than interferon alone and sometimes resulted in complete clearance of hepatitis C virus. In 1998, Judie started on the combination therapy, adding five tablets of ribavirin per day to her regular regimen of injections of interferon three times weekly. Within a month, it was clear that the combination was working better than interferon alone. "The therapy controlled all my symptoms, alleviated my vasculitis and cryoglobulinemia, brought my liver enzyme levels back to normal for the first time in years, and improved the health of my liver in general," says Judie.

A liver biopsy taken after a year of the combination showed a marked decrease in the inflammation and scarring of the liver.

Unfortunately, Judie was not cured of her hepatitis C. Even though the liver tests were normal, the hepatitis C virus could still be detected in low levels in the blood. At the same time, she was having real difficulties tolerating the combination therapy over the long term. "I developed anemia, and all I could manage was surviving at work and cooking dinner at home. I was always exhausted."

Having come this far, Judie accepted enrollment in yet another research protocol. She stopped the interferon injections but continued on the ribavirin by itself, so called ribavirin "monotherapy." Ribavirin would not make the virus go away, but it might block its harmful effects, thus providing a bridge until more effective therapies become available that might totally eradicate hepatitis C. The question being asked in the experimental study was whether ribavirin by itself could control the cryoglobulinemia. The idea of finally getting off interferon appealed to Judie.

In the next six months, her liver enzymes remained normal, her arthritis and rash did not return, and her anemia slowly improved. Importantly, she was feeling much better than in years past. Having stopped interferon after almost five years of continuous treatment, "I feel as if I have my life back," says Judie. "I'm extremely grateful and pleased for the treatment I received at NIDDK. I was treated with a great deal of respect and understanding."

Not everyone infected with hepatitis C has symptoms. But those that do might:

- **Feel tired.**
- **Feel sick to their stomach.**
- **Not want to eat.**
- **Have stomach pain.**
- **Feel joint aches and pains.**

Judie is a board member of the District of Columbia Chapter of the American Liver Foundation, an advocacy group that raises funds for liver research and provides people with information on liver diseases, including hepatitis C, through its hotline and educational programs. She remains optimistic that recent clinical studies show that 40 percent of individuals treated with the combination therapy she has taken have been cured. Her case suggests that ribavirin monotherapy may be able to temporarily improve hepatitis C. Current research supported by NIDDK is focused on making therapy easier, safer, and less costly and on raising the cure rate to 100 percent.

"This disease seems to be more tenacious in those of us infected with a certain viral genotype and who have lived with it the longest," Judie says. "Early detection and treatment seem to be the key." She is determined to remain as healthy as possible until an effective treatment or cure is discovered for her particular variety of the disease—which has been unusually resistant to therapy. Until then, Judie remains dedicated to helping others learn to live with hepatitis C and its treatment.

Beware of Bacteria on Food!!!

Undercooked food can bring rapid illness and death to both children and adults. When harmful bacteria are left on food after it is improperly washed or cooked, grave foodborne illness can result. Two serious diseases usually caused by foodborne bacteria are hemolytic uremic syndrome and cholera. NIDDK-supported researchers are hard at work trying to gain new knowledge that would lead to more effective ways to treat and prevent both diseases.

Hemolytic uremic syndrome (HUS) is a range of kidney diseases caused when a strain of bacteria called *Escherichia coli* O157:H7 releases shiga toxin into the bloodstream. This toxin causes dangerous damage to the kidneys, whose millions of tiny blood vessels filter the body's wastes from the blood. When these blood vessels are damaged, blood clots form and prevent blood filtration. The kidneys can ultimately fail, depriving the body of other kidney functions, including proper maintenance of red blood cells and platelets. Other possible variations of the syndrome thus include anemia, or reduced numbers of red blood cells, and blood-clotting problems due to reduced platelet numbers. HUS frequently occurs in children after an incidence of gastrointestinal infection. A critical research issue is why some children develop HUS following these infections while others do not. Past observations have suggested that antibiotics used to treat gastrointestinal infection may cause *E. coli* bacteria to release shiga toxin. Following this premise, researchers conducted an epidemiologic study which showed that the use of antibiotics to treat children with gastrointestinal infections was indeed linked to an increased chance of developing HUS. Because some cases of HUS develop even in the absence of antibiotic treatment, a full understanding of HUS will require researchers to identify other causes of bacterial shiga toxin release.

Another deadly disease that can be caused by foodborne bacteria is cholera. A severe, infectious diarrhea, cholera is caused by the bacterium, *Cholera vibrio*. Three million children die each year from infectious diarrhea, and the majority of cases are in developing countries. Cholera victims suffer from dehydration and exhaustion when fluid and minerals are lost. In addition to having diarrhea due to increased fluid secreted by the small intestine, victims of cholera infection also demonstrate decreased fluid absorption in the large intestine. Previous studies have shown that short-chain fatty acids can increase the amount of fluid absorbed by the large intestine. It is also known that short-chain fatty acids are produced when the large intestine ferments undigested starch. Based on this knowledge, researchers proposed to reduce the duration of diarrhea by treatment with digestion-resistant starch, in order to produce greater fluid absorption in the large intestine. An experiment compared results of standard treatment with glucose and minerals alone to treatment that included the addition of digestion-resistant starch to the standard regimen. The researchers found that treatment with digestion-resistant starch was able to significantly reduce the duration of diarrhea experienced by cholera victims. This new, effective treatment has the potential to reduce diarrhea-related deaths worldwide.

Ramakrishna BS, Venkataraman S, Srinivasan P, Dash P, Young GP, and Binder HJ. Amylase-resistant starch plus oral rehydration solution for cholera. *New Engl J Med* 342(5):308-313, 2000.

Wong CS, Jelacic S, Habeeb RL, Watkins SL, and Tarr PI. The risk of hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *New Engl J Med* 342(26):1930-1936, 2000.

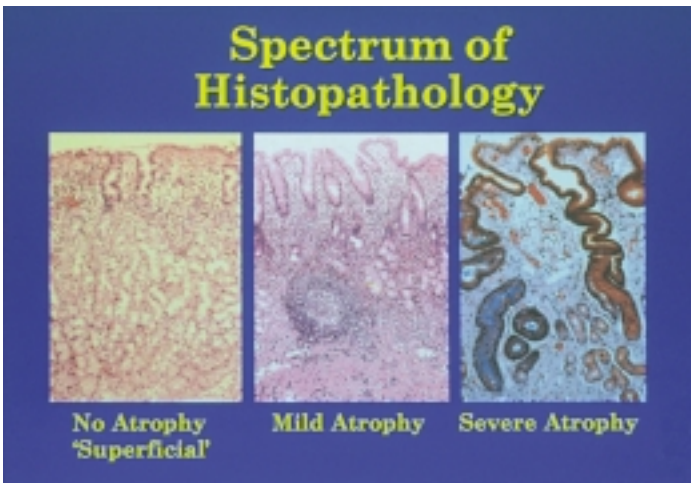
Fighting Peptic Ulcer Disease

Many individuals who develop peptic ulcer disease or a severe form of stomach inflammation known as atrophic gastritis and/or stomach cancer can trace their conditions to a bacterium known as *Helicobacter pylori*. This bacterium causes one of the more common infections of man. Worldwide, more than 50 percent of humans are chronically infected with *H. pylori*. In the U.S., 25 percent of children between the ages of six and nine are infected.

Once, ulcer disease and related pain were thought to be provoked by spicy foods or stress. Treatment consisted of acid-reducing drugs, which cured the ulcers over a period of four to eight weeks; however, the disease would frequently recur within 18 to 24 months. Thus, the former prevailing dogma was "no acid, no ulcer," and "once an ulcer, always an ulcer." The treatment of ulcers was revolutionized by the discovery that eradication of the infection-causing bacterium *H. pylori* can heal peptic ulcers and significantly reduce their recurrence. This

discovery resulted in a new paradigm for addressing the cause of chronic diseases.

Detecting active ongoing infection with *H. pylori* has been made far easier with the Food and Drug Administration's approval of the urea breath test—a noninvasive and inexpensive test developed by NIDDK-funded researchers. This safe and very effective diagnostic tool confirms the presence of the bacterium and helps to tailor appropriate treatment. It is now recommended that all patients with peptic ulcer disease be treated with antibiotics and antisecretory agents once the bacterium is detected. It is estimated that a treatment strategy of antibiotics and antisecretory agents should eliminate 80 to 90 percent of *H. pylori*-related peptic ulcer disease in the U.S. A question remains, however, regarding whether all patients with ulcer-like symptoms should be screened for



Spectrum of *Helicobacter pylori* gastritis diagnosis. The bacterium *H. pylori* can cause infection and damage to the lining of the stomach eventually causing loss of normal stomach cells or "atrophy." Photomicrographs show different degrees of gastric damage and atrophy associated with infection. Photo: American College of Gastroenterology.

H. pylori and treated with antibiotics. Another question is whether healthy people should be screened for infection and treated to prevent possible future peptic ulcers or gastric cancer. These are unknowns that require further basic and clinical research studies.

The frequency of *H. pylori* infection among Americans and the mode of transmission of this disease have not been defined. To shed light on this issue, researchers analyzed a large, population-based group of serum samples from persons in the U.S. for antibodies to *H. pylori*. They then correlated results with demographic factors including sex, age, race and socioeconomic status. The prevalence of *H. pylori* infection increased with age and was far higher in Mexican Americans (58 percent) and non-Hispanic blacks (51 percent) than in non-Hispanic whites (27 percent). The disparities in frequency of infection appeared to be related to socioeconomic class and country of origin. The mode of spread could not be proven by this cross-sectional study, but the results suggested that poor hygiene and crowded living conditions during childhood were associated with a greater likelihood of infection.

Immunization is considered a possible approach to eliminating the *H. pylori* bacterium in high-risk populations. Experiments using immunization in a mouse model have successfully provided high rates of protection against infection. These studies inhibited urease, a protein produced by the bacterium, which facilitates host colonization by neutralizing stomach acid. At the present time, urease seems to be the most promising target for vaccine development.

The fight against *H. pylori* is also being aided by the sequencing of the complete bacterial genome, which will permit its further assessment and characterization. This knowledge will help direct investigations of how this organism leads to ulcer disease, chronic stomach inflammation and stomach cancer. It will also propel the development of new preventive and treatment approaches, including vaccines and other drugs.

Everhart JE, Kruszon-Moran D, Perez-Perez GI, Tralka TS, and McQuillan G. Seroprevalence and ethnic differences in *Helicobacter pylori* infection among adults in the United States. *J Infect Dis* 181(4):1359-1363, 2000.

Battling Digestive Diseases in Childhood: Inflammation in the Immature Gut

As the incidence of premature births increases at earlier stages of pregnancy, a physician who cares for newborns grapples with an immature gastrointestinal tract (GI) unprepared for bacterial assault. Now that respiratory distress syndrome in premature infants is treatable, the major medical challenge in newborn intensive care nurseries becomes necrotizing enterocolitis (NEC), an inflammatory disease that causes tissue damage in the small intestine and colon. NEC is a worldwide problem that occurs after birth, usually during the first or second week of life. NEC is also the most common gastrointestinal emergency in neonatal intensive care units. It is characterized by the triad of abdominal distension,

gastrointestinal bleeding, and air in the intestinal wall. In addition, infants with severe NEC may have air within the portal vein that carries blood from the digestive organs to the liver. Due to the continuing increase in the survival rate of low-birth weight infants, it is expected that NEC will be a significant public health problem with substantial morbidity and mortality.

Bacterial colonization of the gastrointestinal tract is believed to be necessary for NEC to develop because the disease cannot be reproduced in sterile animal models. Strong evidence suggests that the progression of NEC is closely linked to introducing formula feeding of premature infants at a time when bacteria initially colonize the newborn intestine. NEC is rare among infants fed breast milk alone. Investigators have speculated that NEC develops in part due to an immature intestinal immune response to a disease-causing microbe. For this reason, NIDDK grantees chose to study the response of immature human cells to inflammatory stimuli using the pro-inflammatory protein IL-8 to influence the course of the immune response in immature *versus* mature human small intestine. They found that the inflammatory response in cells of premature infants at a time when NEC occurs is related to an excessive IL-8 stimulation following initial colonization of the intestine with disease-causing organisms. The researchers concluded that an inappropriate response of the immature gut to oral feedings and colonization of bacteria represents a developmental risk factor for NEC.

The results of this investigation mark a starting point for dissecting out the balance of pro- and anti-inflammatory signals generated by the intestinal lining in response to inflammatory stimuli. Understanding this balance is critical to mounting an effective response to inflammation and to preventing chronic inflammation. Further research is needed to define the exact steps involved in regulating this process. Much needs to be done to clarify the disease process of NEC, as well as the appropriate preventive measures.

To fuel the research agenda, the NIDDK recently held a workshop on "Motility of the Digestive Tract." Recommendations from the workshop set in motion publication of a proposal to encourage basic and clinical research to identify effective diagnostic modalities and treatment interventions for motility disorders in adults and in children. Among the relevant research topics suggested was identification of factors that regulate the development and plasticity of intestinal cells, as well as inflammatory cells that reside in the normal bowel. Another area of focus was the migration of inflammatory cells in the gut wall. The NIDDK will continue to stimulate research in this important sphere of inquiry in the hope that highly innovative research studies applying state-of-the-art molecular techniques will enhance knowledge of the underlying cellular and molecular mechanisms for motility disorders in infants and children, as well as adults. The Institute will seek research studies of the enteric nervous system, the role of ion channels and interstitial cells of Cajal in motility, and the interaction of the neuromuscular apparatus of the gut and the immune system. In addition, the NIDDK will encourage research evaluating electrophysiological studies of the smooth muscle of the gut, as well as biologic markers for hypersensitivity and brain imaging. An important area of emphasis will be the development of clinical trials to evaluate pharmacological and non-pharmacological approaches to the treatment of functional bowel disorders, cyclical vomiting syndrome, constipation, and fecal incontinence.

Nanthakumar NN, Fusunyan RD, Sanderson I, and Walker WA. Inflammation in the developing human intestine: A possible pathophysiologic contribution to necrotizing enterocolitis. *Proc Natl Acad Sci USA* 97(11):6043-6048, 2000.

New Clues to Inflammatory Bowel Disease

Ulcerative colitis and Crohn's disease are the two most important forms of inflammatory bowel disease (IBD) and represent the major cause of morbidity from chronic intestinal illnesses. The onset of IBD is most often in adolescence and young adulthood and may be followed by devastating long-term consequences. These include malnutrition and growth retardation; compromise of employment and social activities; and an increased risk for intestinal cancer. Over the last two decades research has focused on the cell biology of the intestinal lining, genetic predisposition to IBD, the role of inflammatory mediators in the disease, new animal models, and new therapies. (See accompanying "Story of Discovery" on IBD.)

Preventing the intestine from having an inflammatory response to non-infectious bacteria is vital to maintaining gut health. In a recent study, investigators found that the normal microflora in the gut use molecular pathways of

the epithelial cells that line the intestine to prevent an inflammatory response in the host. The gut environment contains a variety of harmless microflora and bacterial pathogens that coexist and may either invade the mucosa or produce toxins that damage it. To adapt to this potentially hostile milieu, the gut epithelial cells have evolved strategies that provide the intestine with both an active immunologic and anatomic barrier. Researchers have shown that a non-infectious strain of *Salmonella* is able to interfere with activation of the transcription factor in gut epithelial cells and thus with expression of genes involved in the immune response. The bacteria achieve this by blocking degradation of an inhibitor that binds to and captures a transcription factor in the cell's cytoplasm. Once the inhibitor is degraded, this factor is released and moves to the nucleus where it switches on target genes involved in inflammation. The investigators propose that, in this way, the normal gut microflora are able to induce a form of tolerance in gut epithelial cells despite exposure to a variety of bacteria. Future studies need to focus on how an anti-inflammatory state maintained by non-infectious bacteria is overcome to enable an immune response against infectious bacteria. Other questions relate to the significance of different coexisting bacteria in effecting transcription factor activation. Non-infectious organisms, or probiotics, may be effective in some patients with IBD. While their mechanism of action is not clearly understood, it may be that certain bacterial species are able to avoid activating the transcription factor and thus subdue the host's inflammatory response. Dysregulated interactions between microbe and host may underpin not only IBD, but also many other poorly understood chronic inflammatory disorders of the gastrointestinal tract.

A long range plan in IBD research has guided NIDDK on a path that has led to an approximate six-fold growth of the IBD research grant portfolio from 1989 to the present. IBD is an area of focus in three of the Institute's Digestive Disease Research Centers. Related topics of study in other NIDDK centers include liver diseases and disorders, gastrointestinal (GI) biology, digestive diseases, ulcer disease and gastrointestinal hormones. Several new initiatives are planned that include efforts to create an IBD genetics consortium in follow-up to a recent scientific meeting. Two new clinical research networks are also planned, one in adult and one in pediatric IBD. Moreover, the Institute will continue to augment research training approaches to encourage growth in IBD research.

Neish AS, Gewirtz AT, Zeng H, Young AN, Hobert ME, Karmali V, Rao AS, and Madara JL. Prokaryotic regulation of epithelial responses by inhibition of I-kappa B-alpha ubiquitination. *Science* 289(5484):1560-1563, 2000.

Xavier RJ and Podolsky DK. How to get along–friendly microbes in a hostile world. *Science* 289(5484):1483-1484, 2000.

STORY OF DISCOVERY

Inflammatory Bowel Disease

The inflammatory bowel diseases (IBD) known as Crohn's disease (CD) and ulcerative colitis (UC) affect nearly one million Americans. Typical symptoms of IBD include abdominal pain, fever, watery or bloody diarrhea, weight loss, and fatigue. Both forms of IBD are chronic illnesses that typically affect children and young adults and have major negative impacts on their health and quality of life. Traditional therapy for IBD has consisted of immunosuppressive and anti-inflammatory drugs, antibiotics, and drugs to relieve the pain, fever, and other overt symptoms of the disease. Unfortunately, about one third of patients do not respond to medical treatment, and—in patients who do respond—remission is usually followed by relapse. Many patients ultimately require one or more surgeries to alleviate their symptoms.

Research is yielding new clues about the common final manifestation of IBD: chronic inflammation of the intestinal tract. The body's immune system is designed to identify and eliminate foreign invaders, generically termed "antigens." Inflammation is a complex response to an antigen, which includes increased blood flow to the affected region and an influx of cells to defend the body. This process is facilitated in part by the production of cytokines, proteins released by cells to alert the body to the presence of a threat and that may either promote or inhibit inflammation. Important pro-

inflammatory cytokines include gamma interferon, some members of the interleukin (IL) family, and tumor necrosis factor (TNF)-alpha. As the cells of the immune system destroy the antigen, the degree of the inflammatory response decreases and the injured area subsequently undergoes repair and recovery.

Under normal conditions, a balance exists between signals that promote inflammation and those that inhibit it. In patients who suffer from IBD, however, this balance is perturbed and pro-inflammatory signals predominate in the intestinal tract, leading to chronic inflammation and resultant tissue damage. While the trigger for this disturbance is unknown, it seems to arise from an abnormal reaction by the immune system to the bacteria normally present within the gut. A likely explanation for this aberrant immune response is that susceptible individuals inherit a genetic predisposition to IBD and possess an immune system less able to distinguish between benign and threatening stimuli. A major goal in the treatment of IBD is to induce and sustain remission over time, thereby limiting tissue damage and improving the quality of life for affected individuals. Drugs currently used to treat IBD fall into one of two general categories. One group acts quickly to relieve symptoms but is unsuitable for long-term use, owing to undesirable side effects. A second group of drugs is effective at maintaining remission over time, but is slow to act, thereby having limited usefulness in treating acute disease. Because of these limitations, researchers seek a fuller understanding of IBD at the molecular level, in the hope of identifying novel targets for new therapies.

Animal models of IBD have provided a wealth of new information about disease onset and progression. Historically, researchers induced IBD in animals by supplementing their diet with chemicals that irritated the lining of the bowel, producing symptoms reminiscent of human IBD. With the advent of molecular genetics, however, more sophisticated models of IBD have emerged. These models have revealed new insights into the origins of IBD and the roles played by pro- and anti-inflammatory cytokines. For example, mice engineered to overproduce TNF-alpha, a potent pro-inflammatory cytokine, exhibit severe intestinal inflammation that closely resembles human Crohn's disease. Mice lacking IL-10, an anti-inflammatory cytokine, also develop widespread intestinal inflammation. Together, these findings indicate that disequilibrium in the balance between the levels of pro- and anti-inflammatory signals, resulting from either increased production of factors that promote inflammation or the absence of factors that inhibit it, can give rise to conditions that closely resemble IBD. Interestingly, in IL-10 deficient mice, the severity of IBD seems to be related to the presence of bacteria within the bowel, because mice housed in germ-free conditions or treated with anti-bacterial drugs develop more limited disease than do mice that are raised in a conventional environment. These studies support the hypothesis that IBD may arise from an inappropriate immune response, facilitated by a permissive genetic context, to otherwise benign environmental factors. The illness does not develop in genetically normal mice nor in mutant mice housed under special conditions.

These laboratory insights are currently being translated into novel therapies that target the molecular mediators of inflammation. Multiple clinical trials have examined the benefits of inhibiting pro-inflammatory stimuli or boosting the levels of anti-inflammatory signals in Crohn's disease. In these studies, the effectiveness of the experimental treatment is assessed using the Crohn's Disease Activity Index (CDAI), a numerical score that reflects multiple aspects of the disease. Scores of 200 to 400 indicate moderately active disease, while scores below 150 denote remission. "Clinical response" to treatment is usually defined as a decrease of 70 points or more in the index, which may not necessarily indicate remission. One strategy involves targeting TNF-alpha, perhaps the prototypic pro-inflammatory cytokine. In active Crohn's disease, a single injection of infliximab, an antibody that inactivates TNF-alpha, promotes a clinical

response in two-thirds of patients and remission in approximately one-third. IL-10 has also been investigated as a potential therapy. In a small trial, disease activity scores were 50 points lower and remission rates were twice as high in Crohn's patients who received IL-10 for three weeks compared to those who did not.

As direct mediators of the immune response and inflammation, cytokines are obvious targets for novel IBD therapies. However, unexpected insights into the molecular causes of IBD have come from recent studies of the PPAR-gamma gene, which is not a cytokine. It is a member of a family of proteins known as transcription factors that regulate which genes are turned on or off within a given cell. PPAR-gamma was originally characterized as a protein that regulated metabolism and promoted the development of fat cells, and was investigated for its role in the development of diabetes. Surprisingly, cells of the large intestine also express PPAR-gamma, where it can inhibit the production of pro-inflammatory cytokines. This discovery has led to the consideration of agents that activate PPAR-gamma, and thereby reduce the levels of pro-inflammatory cytokines, as possible therapies for IBD. Several lines of experimental evidence support this reasoning. In a cell culture system, both naturally occurring and synthetic PPAR-gamma activators inhibit the ability of intestinal cells to produce pro-inflammatory cytokines. Furthermore, in a mouse model of IBD, a significant decrease in severity of disease is noted when the mice are treated with synthetic PPAR activators. These results suggest that therapies targeting PPAR may be an effective component of an anti-IBD regimen. A pilot clinical trial, supported by NIDDK, is now under way to investigate this possibility.

All of these therapeutic strategies are designed to diminish the inflammatory response in order to relieve the symptoms of IBD. Future improvements in treatment of IBD, however, are likely to come from the identification of the genetic lesions that initially give rise to the disease. To identify genes that may be involved in a given disorder, scientists analyze DNA from genetically similar people, such as large families or members of relatively homogeneous ethnic groups, and look for a correlation between specific chromosomal segments and the occurrence of the disease. Using this approach, researchers have noted several genetic regions that seem to correlate with the development of IBD. The identification of multiple genetic loci on different chromosomes—some for Crohn's disease, some for ulcerative colitis, and others for both—suggests that there is unlikely to be a single underlying defect responsible for all forms of IBD. Although the identification of individual genes responsible for the development of IBD is years away, this information nevertheless represents an important first step in understanding the underlying genetic causes of IBD.

Ultimately, all these insights will be synthesized to provide a more complete base of knowledge about the causes of and potential treatments for IBD. While drugs focusing on cytokines such as TNF-alpha and IL-10 are effective in some patients, these novel therapies do not represent cures, because symptoms of the disease return after treatment is discontinued. To address this problem, researchers are currently conducting important work to investigate the possibility that combinations of agents—each targeting a different component of the immune response or a different facet of the disease—will prove more effective in the management of IBD than any single therapy. Critical insights into the origins of IBD will likely come from the identification of genes responsible for disease predisposition. In the future, these advances will lead not only to a clearer understanding of the disease, but also to new targets for drug development. Furthermore, genetic diagnosis should permit earlier detection of individuals at risk and should facilitate strategies to prevent the occurrence of IBD.

PATIENT PROFILE

Ken Rosenau – Crohn's Disease

Ken Rosenau, a 45-year-old trial attorney, remembers being the smallest kid in high school. "When I was 15 they wanted me to play the part of an 8-year-old in the school play," he says. What they—his family, friends, doctors, and Ken, himself—didn't know at the time was that he was suffering from Crohn's disease, a serious, recurrent inflammatory bowel disease (IBD) that, among many other devastating affects, can delay development and stunt growth in children. For years, Ken suffered abdominal pains and diarrhea. "Everyone, including my doctors, thought I was either faking or exaggerating my symptoms," says Ken. During one three-day period, as a result of severe diarrhea, he lost seven pounds. "My doctor said it was probably just a flu virus running through me." It was shortly after he graduated from college and was being operated on for several infected abscesses on his intestinal wall, that he was diagnosed as having Crohn's disease. Over the years, Ken has undergone several surgeries, including a triple bowel resection and temporary colostomy. These procedures allow waste to be removed from the body through a hole made in the abdominal wall into a pouch, which is emptied by the patient as needed. Today, Ken controls his Crohn's disease through diet and anti-inflammatory drugs.

Ken Rosenau suffered for years before his Crohn's disease was diagnosed. Although Ken readily admits that Crohn's is "a very difficult disease to live with," recent treatment advances have improved the quality of life for Ken and many other patients.

Although there is no cure for Crohn's disease, research supported by the NIDDK is succeeding at developing treatments to control inflammation, correct nutritional deficiencies, and relieve symptoms such as abdominal pain, diarrhea, and rectal bleeding, so that patients like Ken, and the 500,000 other Americans who suffer from Crohn's disease and other inflammatory bowel diseases, can lead more normal lives.

About Crohn's Disease

Crohn's disease usually affects the lower part of the small intestine, called the ileum, but it can occur in any part of the digestive tract, from the mouth to the anus. The inflammation extends deep into the lining of the affected organ, causing pain. It can also make the intestines empty frequently, resulting in diarrhea.

The disease affects men and women equally, seems to run in families, and manifests itself variably in different people with respect to location and severity. About 20 percent of people with Crohn's disease have a blood relative with some form of IBD. In Ken's case, his uncle suffers from ulcerative colitis, which differs from Crohn's in that it causes inflammation and ulcers in the large rather than the small intestine. The causes and mechanisms that lead to these diseases are not clear. However, the most popular theory is that a defect in the body's immune system reacts to bacteria colonizing the intestine, causing ongoing inflammation of the intestinal wall. There is no evidence that Crohn's disease or colitis are caused by emotional distress or sensitivity to foods or food products, but it is believed that these factors may worsen symptoms in some people.

Living with the Disease

People with Crohn's disease often must learn to live with constant nausea, dehydration, and abdominal and rectal cramping, as well as diets limited to soft foods and nutritional supplements. "I'm paranoid about what I

eat," says Ken, who adds that when he eats "something wrong, it destroys a couple of days of my life. Between sleep deprivation, nausea and diarrhea, things get pretty rough for awhile." During the course of his disease, he has also suffered several internal fistulas. Fistulas are sores or ulcers that tunnel through the affected inflamed area into surrounding tissues, such as the bladder or skin, or to another section of the bowel. They can only be corrected by surgery.

Because of inflammation in the intestinal wall, the body also does not absorb food properly. As a result, malnutrition and growth retardation are associated with Crohn's disease, as well as increased risk for intestinal cancer. Ken says he needs to eat approximately 3,500 calories a day (2,000 calories are required to sustain an average-size man) just to maintain daily nutritional requirements.

CROHN'S DISEASE FACTS

- **Crohn's disease is an inflammatory bowel disease (IBD) that causes inflammation in the small intestine.**
- **Approximately 500,000 people in the U.S. suffer from IBD, the majority of whom have Crohn's disease or ulcerative colitis, which differs from Crohn's in that it causes inflammation and ulcers in the large rather than the small intestine.**
- **Crohn's disease symptoms include abdominal pain, diarrhea, rectal bleeding, weight loss and fever.**
- **Complications of Crohn's disease include blockage of the intestines, as well as sores, or ulcers that tunnel through affected areas into surrounding tissue such as the bladder or skin.**

Ken is fortunate, however, in that he hasn't suffered any of the other complications often associated with Crohn's disease, including arthritis, skin problems, inflammation in the eyes or mouth, kidney stones or gallstones, or liver disease. He has undergone several surgeries. Some people with Crohn's need to have their entire colon removed permanently. Most of these patients go on to live relatively normal, active lives. However, some are so psychologically devastated by this procedure, as well as the other complications brought on by Crohn's disease, that they require counseling to deal with their feelings.

Research Leads to More Effective Treatments

Although surgery can help people with Crohn's, it cannot cure the disease. In fact, the disease often recurs after surgery. As a result, research has focused on drug therapies and the cell biology of the intestinal lining to develop medications that will slow or arrest the disease process. For example:

- The mainstay for treatment of severe attacks of Crohn's disease has been medications that depress the immune system, which seems to be overactive in this disease. The usual medications in this class are corticosteroids, such as prednisone. While prednisone has a marked effect on Crohn's disease, it also has significant side effects that become troublesome with prolonged therapy. These side effects include thinning of the skin, osteoporosis, high blood pressure, weight gain, diabetes, and glaucoma.

- A new corticosteroid, called budesonide, recently has been identified. Budesonide appears to be as effective as other corticosteroids but causes fewer side effects.

- Recent advances in research have provided new insights into the role of immune system cells and their cytokines in chronic intestinal inflammation. Cytokines are regulatory proteins that play a critical role in the course of many autoimmune diseases, including Crohn's disease. Cytokines can have pro-inflammatory or anti-inflammatory effects.

- Research has led to the development and FDA approval of the drug infliximab, an anti-tumor necrosis factor (anti-TNF-alpha). TNF-alpha is a cytokine that may be responsible for the inflammation of Crohn's disease. Anti-TNF-alpha is an antibody that finds TNF-alpha in the bloodstream, binds to it, and removes it before it can reach the intestines and cause inflammation. Studies have shown

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that anti-TNF-alpha seems particularly helpful in closing fistulas.

- Researchers are studying the effectiveness of IL-10, another cytokine, as well as other immunosuppressive drugs, such as methotrexate and cyclosporine, to treat Crohn's disease. IL-10 may have anti-inflammatory effects opposite to those of TNF-alpha.

- Some research suggests that antibiotics now used to treat the bacterial infections that often accompany Crohn's disease might also be useful as a primary treatment for active Crohn's disease.

Ken and thousands of others suffering from Crohn's disease and other forms of IBD have benefitted greatly from such research. For example, in the 1980s, the anti-inflammatory drug, prednisone, helped to treat Ken's inflammation. Today, Ken takes a milder, aspirin-like compound with minimal side effects to prevent the recurrence of inflammation.

Because of research conducted and supported by the NIDDK, Ken is able to lead a more normal lifestyle. He's married, is a successful attorney who specializes in bioethics and children's rights, and is a board member of the District of Columbia affiliate of the Crohn's and Colitis Foundation of America. As an attorney, Ken is frequently appointed guardian for clients who cannot make health care decisions for themselves. "The upside of having Crohn's disease is that it has made me knowledgeable about health care issues and much more empathetic to my clients," he says. At the same time, Ken is hoping that further research into Crohn's disease will continue to improve his life, and the lives of thousands of others who suffer from this and other forms of IBD.

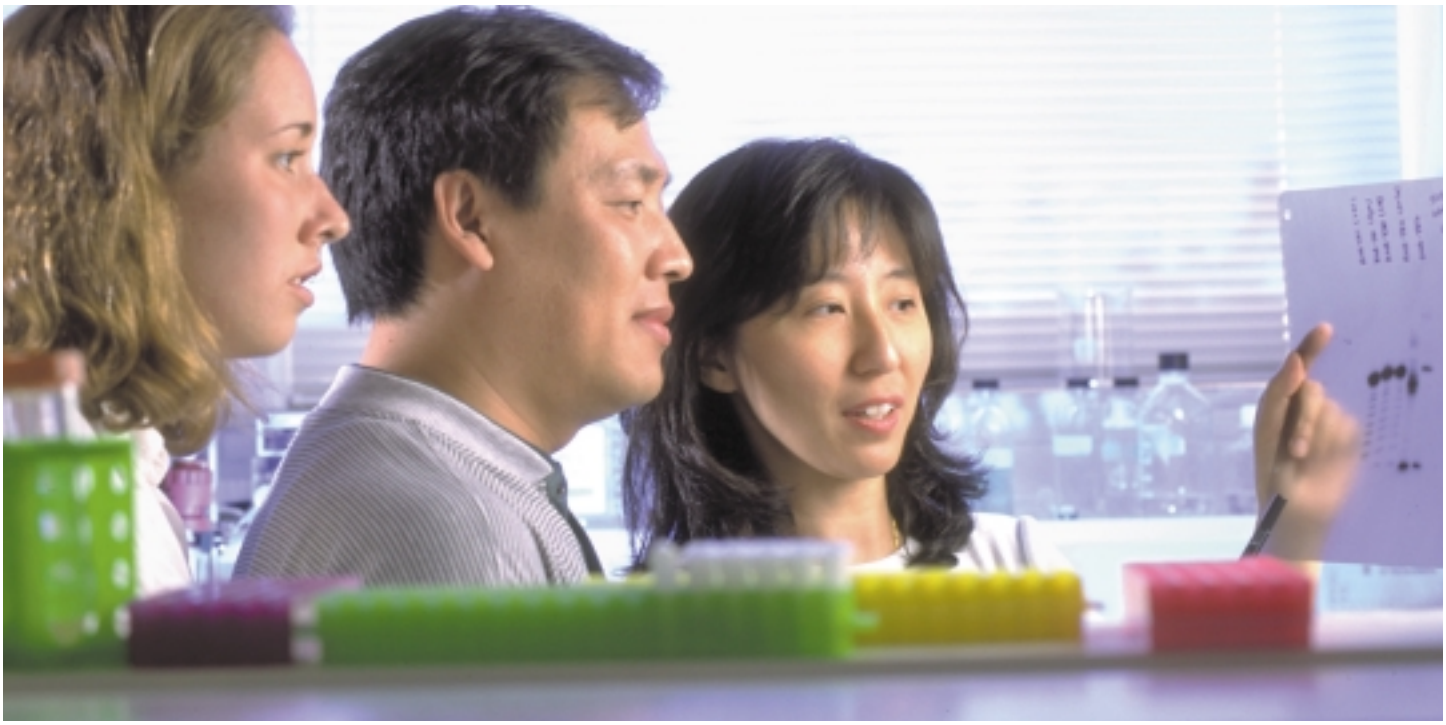

Photo: Mr. Richard Nowitz.

Stopping Gastrointestinal (GI) Cancer

Gastric cancer is a leading cause of morbidity and mortality worldwide. As in many types of tumors, the development and progression of gastric cancer is a multi-step process. Researchers recently established that one mechanism of tumor progression is the inactivation of the signaling pathway of transforming growth factor (TGF)-beta. TGF-beta is a regulatory protein with diverse biological activities. The tumor suppressor gene *Smad4* is the central mediator of this pathway. In research studies, mice lacking Smad4 protein exhibited impaired embryonic membrane formation and tissue differentiation and died during embryonic development. Researchers next assessed the tumor suppressor function of *Smad4* in mice lacking one of a pair of normal genes. These mice developed hyperplasia—an abnormal increase in the number of normal cells—within certain portions of the gastrointestinal (GI) tract. Tumors rarely resulted from hyperplasia of the fundal region, or the portion of the stomach to the left and above the entrance to the esophagus. On the other hand, hyperplasia in the antrum, that portion of the GI tract between the body of the stomach and the opening to the intestine, eventually developed into tumors as the mice aged. However, loss of the remaining normal *Smad4* gene was detected in these animals only in the later stages of tumor progression. These data indicate that *Smad4* is a major tumor suppressor gene in the GI tract, especially in the stomach, and that loss of one normal copy is sufficient for tumor initiation. Data also showed that over-production of TGF-beta and other proteins was associated with increased cell number and eventual development of tumors. These findings demonstrate the value of this model for screening factors that may promote or prevent the formation of tumors.

Most deaths from colorectal cancer should be preventable with currently available screening methods. The U.S. Preventive Services Task Force, the Agency for Health Care Policy and Research, and medical organizations broadly endorse annual testing for fecal occult blood and periodic sigmoidoscopy after the age of 50 years for persons at average risk of colorectal cancer. While sigmoidoscopy allows the doctor to look into the rectum and lower part of the colon through a flexible tube inserted through the anus, colonoscopy permits a comprehensive evaluation of the entire large bowel. An alternative to colonoscopy or sigmoidoscopy is barium-enema evaluation. The barium enema x-ray involves putting barium, a chalky solution, into the upper or lower intestines, which shows up white on x-ray film and thus reveals abnormalities in the intestine.

With increasing improved screening methods, why do so many people still die of colorectal cancer? One investigator has suggested that the nature of the problem and the procedures used for screening may account for the lack of compliance with the recommendations. For example, the clinical significance of a polyp in the lower colon is uncertain. In one study, investigators attempted to determine if incidence of lower colorectal polyps was associated with upper colorectal cancer. They compared patients with polyps in the lower colon to those without colon polyps by analyzing data from about 2,000 asymptomatic persons who underwent colonoscopic screening for the first time as part of a program sponsored by their employers. Study results showed that asymptomatic persons 50 years of age or older who have polyps in the lower colon are more likely to have advanced cancer than are persons without lower colorectal polyps. However, the investigators found that almost half the patients with advanced cancers in the upper colon had no lesions in the lower colon. Hence, it was concluded that if colonoscopic screening is performed only in persons with polyps in the lower colon, about half of the cases of advanced tumors in the upper colon will go undetected.

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These recent findings appear to reinforce a growing concern among physicians that flexible sigmoidoscopy for colorectal cancer screening is a less than optimal approach, and physicians should consider carefully whether to promote it. A substantial number of persons have been found to have advanced polyps or carcinomas only in the upper colon, which sigmoidoscopy does not reach. Earlier surveys showed that most lesions were in the lower colon, which would support reliance on sigmoidoscopy for screening. A more recent survey, however, has shown that advanced cancers are more uniformly distributed throughout the colon which confirms that

sigmoidoscopic screening will fail to detect a substantial proportion of asymptomatic colorectal cancers or polyps associated with a high risk of cancer.

Also of mounting concern is a condition known as gastroesophageal reflux disease (GERD). GERD is believed to be a common chronic disorder in the U.S. Its actual prevalence is difficult to establish owing to a dearth of well-conducted epidemiology studies and failure of patients to seek medical attention for symptoms. Typical symptoms of GERD include heartburn and regurgitation. Atypical symptoms include unexplained chest pain, chronic hoarseness, chronic cough, or asthma. The cause and course of GERD are multifactorial. The esophagus becomes inflamed when the backward flow, or reflux, of the stomach contents into the esophagus comes in contact with the esophageal lining for a sufficient time to overcome its defense mechanisms. Those mechanisms include the antireflux barrier, efficient clearing of the acid reflux, and defenses of the cells lining the digestive tract. Left untreated, GERD can result in such complications as inflammation of the esophagus, stricture, hemorrhage, and an abnormal change in the cell surface of the tissue lining the lower esophagus. This change of the flat normal lining of the esophagus to an abnormal columnar-lined covering is defined as Barrett's esophagus, the major risk factor for cancer of the esophagus.

Scientific evidence leaves little question that Barrett's esophagus is associated with GERD. Both animal and human studies confirm that acid, and possibly gastric reflux acting in synergy, cause the most esophageal damage. With the dramatic increase in esophageal cancer in the population, it becomes ever more important to detect Barrett's esophagus; however, screening guidelines remain controversial. While endoscopy is considered the gold diagnostic standard, it is not cost-effective for use in the large number of patients with GERD for identification of columnar-lined esophagus. Clinical trials have shown that patients with reflux esophagitis can remain in remission by effectively suppressing acid. It has been suggested that more aggressive treatment of GERD can prevent complications such as columnar-lined esophagus. The appropriate use of diagnostic endoscopies to identify the columnar-lined esophagus earlier would permit such aggressive antireflux treatment.

Perhaps, as has been suggested, patients with GERD who should undergo endoscopy and be evaluated for columnar lined esophagus might include: (1) patients with a stricture, severe esophagitis, or esophageal ulceration, and (2) patients with a long history of persistent reflux symptoms.

Epidemiologic data have shown that the incidence of cancer in the area of the junction between the stomach and the esophagus is rising at an alarming rate. It now accounts for 50 percent of all esophageal malignancies in the U.S. Because symptoms and endoscopy are unreliable tools for detecting this cancer, tissue sampling is essential. It is not known how useful cytology is to study the origin, structure, function, and pathology of cells. To determine the prevalence of intestinal cancer in the lower esophagus in an adult population with diverse upper gastrointestinal symptoms, researchers invited patients having upper gastrointestinal endoscopy over a six-month period to participate in a prospective study. Clinical data and endoscopic findings were recorded from 155 patients. Cytology and biopsy specimens were obtained from both sides of the normal columnar junction, and the cytology specimens were stained. Results showed that cytology using the stain is not as sensitive and specific as tissue structure for detecting cancer in the lower esophagus. It may, however, prove to be at least as useful as tissue sampling in detecting abnormal development in size, shape and organization of adult cells.

The NIDDK is working with the National Cancer Institute to establish a working group of external advisors to examine the scientific literature on esophageal carcinoma. This group will assess what is known and not known about the risk factors, epidemiology, management and monitoring of this deadly cancer. The group will also consider the area of reflux esophagitis and its potential complications of esophageal strictures and the premalignant lesion, Barrett's esophagus. Based on the recommendations of the working group, initiatives will be developed to address the gaps in scientific knowledge.

The NIDDK recently established a multicenter endoscopy database through an award to support research activities generated by an established endoscopic data center. The new endoscopy database has already developed a plan for studies on Barrett's esophagus. The data center maintains a relationship with a number of

clinical affiliates providing endoscopic reports in multicenter studies.

Finally, with appropriate diagnostic screening methods, the columnar-lined esophagus can be discovered earlier, which would permit aggressive antireflux treatment.

Imperiale TF, Wagner DR, Lin CY, Larkin GN, Rogge JD, and Ransohoff DF. Risk of advanced proximal neoplasms in asymptomatic adults according to the distal colorectal findings. *N Engl J Med* 343(3):169-174, 2000.

Podolsky DK. Going the distance–the case for true colorectal-cancer screening. *N Engl J Med* 343(3):207-208, 2000.

Ter R and Castell DO. Gastroesophageal reflux disease in patients with columnar-lined esophagus. *Gastroenterol Clin North Am* 26(3):549-563, 1997.

Wang HH, Sovie S, Zeroogian JM, Spechler SJ, Goyal RK, and Antonioli DA. Value of cytology in detecting intestinal metaplasia and associated dysplasia at the gastroesophageal junction. *Hum Pathol* 28(4):465-471, 1997.

Xu X, Brodie SG, Yang X, Im YH, Parks WT, Chen L, Zhou YX, Weinstein M, Kim SJ, and Deng CX. Haploid loss of the tumor suppressor *Smad4/Dpc4* initiates gastric polyposis and cancer in mice. *Oncogene* 19(15):1868-1874, 2000.

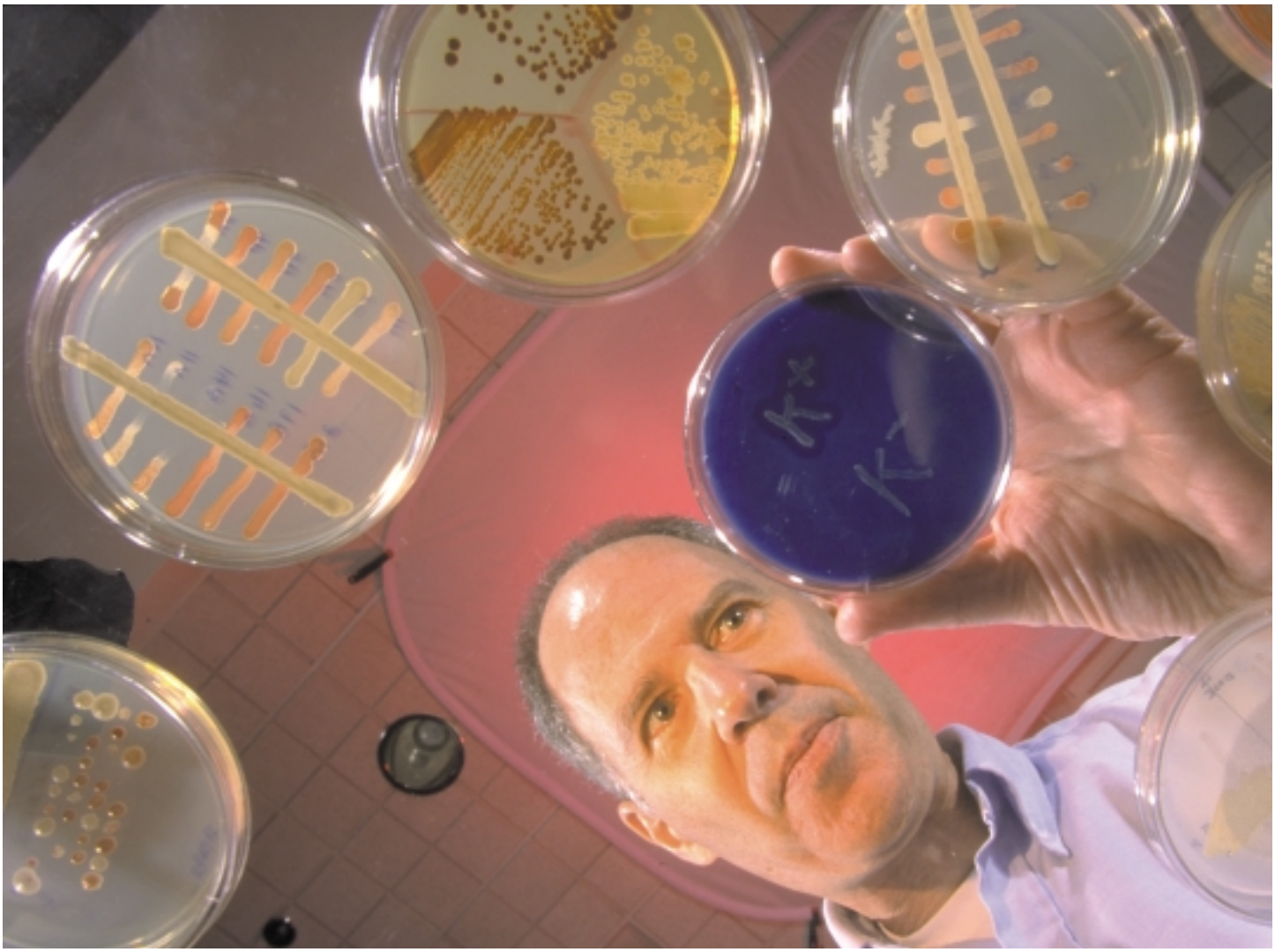

Photo: Mr. Richard Nowitz.