1988

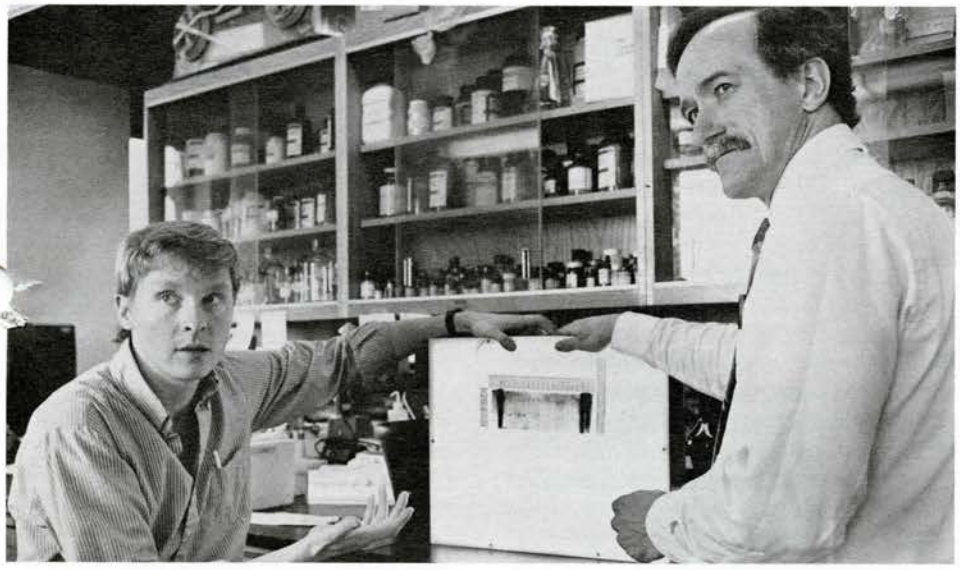
Research in Progress: Fall 1988 v. 9, no. 2

Boston University
School of Medicine

Research in Progress

Volume 9 Number 2
Fall 1988

Molecular epidemiology is enabling BUSM researchers to study, with a view toward creating more effective vaccines, genetic variation in populations of bacteria that cause serious infections of the urinary tract. **See story on page 3.**



Richard M. Niles, Ph.D., right, and graduate student John Stoddard discuss the anti-tumor effects of vitamin A. (Photo by Michael McGovern)

BUSM studies uncover links between vitamin A and cancer

While the debate continues over the protective effects of certain foods against cancer, work is going forward in the laboratory of Richard M. Niles, Ph.D., a professor of biochemistry at Boston University School of Medicine, on the anti-tumor properties of one important dietary requirement—vitamin A.

Niles and his colleagues have a number of experiments under way that are aimed at revealing how at the molecular level retinoic acid—one of the forms vitamin A is converted to in the body—prompts a tumor's undifferentiated cells to return to their normal, differentiated state.

Vitamin A is well understood in terms of the role it plays in vision and the prevention of night blindness. But it also is important in two other areas. One is during fetal development, where a critical concentration of vitamin A is required for normal growth and development; too much or too little results in malformations.

"The second area in which vitamin A is needed is in maintaining epithelial tissues in their normal state of differentiation," explained Niles. (Epithelial cells line body cavities and carry out most of the functions of various organs, such as the secretion of mucus.) "In other words, vitamin A keeps epithelial cells in their proper state so that they can do their job."

continued on page 2

Research on myocardial relaxation leading to new therapies for heart failure

Noninvasive cardiac diagnostic techniques brought into widespread use only in the last 10 years have led to the reassessment of a previously unappreciated kind of heart disease, according to Carl S. Apstein, M.D., a professor of medicine at Boston University School of Medicine and director of the School's Cardiac Muscle Research Laboratory (CMRL).

As many as 30 percent of patients with heart failure symptoms, it now is known, experience shortness of breath and develop pulmonary congestion as a result of an inability of the heart muscle to relax completely between beats (during diastole), rather than from an impairment of the heart's ability to contract and push the blood through the arteries (during systole). Abnormalities of myocardial relaxation mean pressures inside the ventricles remain too high during diastole, impairing blood flow into the chambers and raising the capillary pressures in the lungs, resulting in shortness of breath.

Apstein, who also is chief of cardiology at Boston City Hospital, and his co-workers have been investigating mechanisms of myocardial relaxation in normal and abnormal heart muscle,

continued on page 5

Vitamin A...
continued from page 1

Vitamin A appears to act against the two main hallmarks of cancer—rapid cell growth and undifferentiated character. In most cancer cells, vitamin A halts growth and in many types of tumors, either by itself or in conjunction with another chemical, forces the undifferentiated cells to take on their normal properties. To investigate this connection, Niles is looking at three model systems in culture; two are derived from tumor cells and one from normal cells:

Using a melanoma cell line, Niles is investigating how retinoic acid inhibits cell growth and changes the activity of two key regulatory enzymes involved in growth and differentiation. Cells treated with retinoic acid have more of these enzymes in them than untreated cells and Niles is conducting experiments to see how they carry out the work of inhibiting tumor growth.

In connection with these studies, Niles is working with Judith Campisi, Ph.D., an assistant professor of biochemistry, to understand how retinoic acid supresses the expression of the proto-oncogene c-myc. Cells that are growing rapidly, such as melanoma cells, express c-myc at a high level. Upon exposure to retinoic acid, the expression of the c-myc gene is drastically inhibited—but the question remains, why? Does expression decrease because growth is slowed, or is the decrease of c-myc expression responsible for the slowed growth?

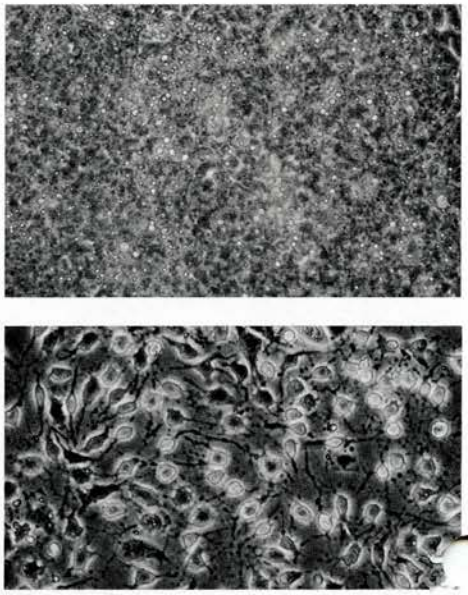
In a second set of experiments, Niles is using a teratocarcinoma cell line derived from mouse testes to understand how retinoic acid works with another compound to transform malignant germ cells into non-malignant cells. It has been shown that these non-malignant cells do not cause tumors when injected back into the mouse. Because teratocarcinomas are derived from germ cells, they have the potential to differentiate into any tissue of the body. Tumors of this type tend to spontaneously differentiate so that a single tumor might contain eye tissue, kidney tissue and hair cells, for example. In this system, cyclic adenosine monophosphate (cAMP) is needed along with retinoic acid to induce normal differentiation.

"We used to think that retinoic acid by itself could drive the differentiation process, but recent re-evaluation has indicated that retinoic acid alters the cells in some way so that they become receptive to cAMP and that it is actually cAMP that drives the differentiation most of the way," said Niles.

Niles is using this cell line to study how this differentiation process occurs at a molecular level, and why the differentiated cells lose their malignancy.

The third system under investigation is a normal cell line derived from the respiratory tract of a hamster. When airway epithelial cells (the ciliated and mucus-producing cells that line the entire respiratory tract except for the terminal air sacs in the lungs) are taken from the animal and cultured, they tend to lose their differentiated function (their ciliated or mucus-secreting characteristics) and grow rapidly until they fill the dish. At that point, the cells re-acquire their normal functions. When epithelial cells are grown with the minimum requirement of retinoic acid and then given supplements at different stages of their growth, Niles has found that the differentiation process can be affected. It appears that if retinoic acid is added at a time when the cells are rapidly growing (without differentiation), differentiation can largely be preserved.

"This is important," said Niles, "because if you put animals on a vitamin A-deficient diet, you can observe that the cells of the airway flatten out and lose their differentiated properties, which is characteristic of precancerous change. You can remove these cells from the animal and culture them with vitamin A and restore their normal shape and function."



Top photo shows uncontrolled growth of F9 teratocarcinoma cells. In bottom photo, cells treated with retinoic acid and cyclic AMP differentiate and become non-carcinogenic. (Photos courtesy of Richard M. Niles, Ph.D.)

In addition to these experiments, Niles is developing a model to study carcinogenesis in normal epithelial cells. "About 75 percent of human cancers are derived from epithelial cells, including cancers of the breast, colon, prostate and lung," he said.

Niles intends to take normal epithelial cells and introduce cancer-causing genes into them. In this way he will be able to determine, first of all, which of a battery of known oncogenes will transform the normal cells into tumor cells. If he can establish this, he then can determine if retinoic acid can prevent or ameliorate this transformation.

Research on how retinoic acid produces its anti-tumor effects received a boost recently with the discovery of a new class of retinoic acid binding protein with a DNA-binding region. "The thinking now is that

these proteins reside in the nucleus and that binding proteins in the cytoplasm act as shuttles to take retinoic acid from the cell membrane to the nucleus. At the nucleus retinoic acid is received by the "new" protein and bound to specific gene sites where it turns off or on a sequence of events to slow growth and induce differentiation," explained Niles.

In collaboration with Abdul Traish, Ph.D., an associate research professor of biochemistry, Niles is chemically synthesizing peptides that represent certain regions of the new receptor proteins and has made antibodies against them. Using the antibodies, Niles and Traish can determine whether or not the proteins are localized in the nucleus. Niles also has a mutant strain of melanoma cell that is resistant to the action of retinoic acid and he will be looking to see if the mutation is in this new class of retinoic acid receptors.

In addition to Campisi and Traish, Niles is assisted by master's degree candidate Betsy Winslow, doctoral candidates Beth Goldstein and Anita Terpstra, and research technicians Barbara Loewy and Kate Brown. Niles' research is supported by the American Institute for Cancer Research; the National Cancer Institute; the National Heart, Lung, and Blood Institute; and Aid for Cancer Research, a Boston-area nonprofit organization that raises funds for cancer research.

—Caroline H. Lupfer

Suggested Further Readings

1. Niles, R.M. et al: Characterization of extended primary and secondary cultures of hamster tracheal epithelial cells. *In Vitro Cell Develop Biol. 24:* 457-463, 1988.
2. Niles, R.M. et al: The effect of sodium butyrate and retinoic acid on growth and CEA production in a series of human colorectal tumor-cell lines representing different states of differentiation. *Cancer Invest. 6:* 39-45, 1988.

Molecular epidemiology yields insights into prevention of bacterial infection

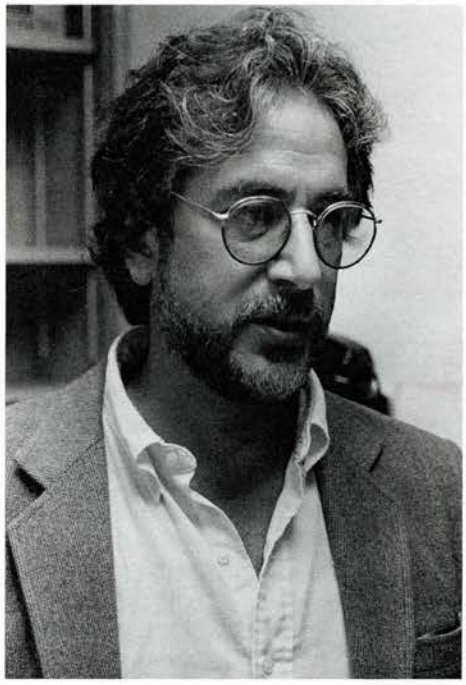
Lower urinary tract infections are a recurring problem for about 10 percent of premenopausal women, and upper urinary tract infections, though less common, are a potentially life-threatening condition for those affected.

Richard Goldstein, Ph.D., a professor of molecular genetics and epidemiology in the Section of Environmental Health of the School of Public Health at Boston University School of Medicine, has spent several years exploring how genetically controlled variations in the molecular structure of bacterial surfaces influence the risk of getting such infections. Work he and his associates have done with the bacteria *Escherichia coli* suggest that variations in the makeup of its surfaces may significantly influence the virulence of those strains that enter the urinary tract.

E. coli normally inhabit the intestinal tract. The strains responsible for urinary tract infections—actually a very small proportion of all *E. coli* found in the gut—do not pose a health threat when in the intestines. If they enter the urinary tract, however, they can be a serious problem.

Goldstein's group studied *E. coli* isolated from various patients, including young girls treated for urinary tract infections at Massachusetts General Hospital, Cleveland Metropolitan Hospital and Boston City Hospital. They were surprised to find that all the isolates differed in their chromosomal structures.

According to Goldstein, this variability appears to result in part because certain key genes that determine a bacterium's virulence occupy widely different sites on the bacterial chromosome, and because DNA sequences within these virulence genes show subtle but crucial degrees of variation. In addition, major portions of the total bacterial chromosome appear to be in flux. The extent of such constant, though low-frequency, rearrangements is such that among *E. coli* strains isolated from several hundred patients with the same type of infection, molecular epidemiologic analyses indicated that no two patients yielded bacteria with an identical order of genes.



Richard M. Goldstein, Ph.D., is studying the role of E. coli in urinary tract infections. (Photo by Bradford F. Herzog)

Insights resulting from this new approach could have profound implications for efforts to create vaccines for urinary tract infections and other bacterial diseases, said Goldstein.

A traditional vaccine stimulates responses only against the inherently limited number of surface molecules

found at a given moment on the organism against which it was made. In light of the enormous genetic variability the Goldstein group has found in *E. coli*—also the major cause of infant mortality due to diarrheal disease—it is clear that all the genetic-mediated changes of surface components need to be characterized to produce useful, multivalent vaccines.

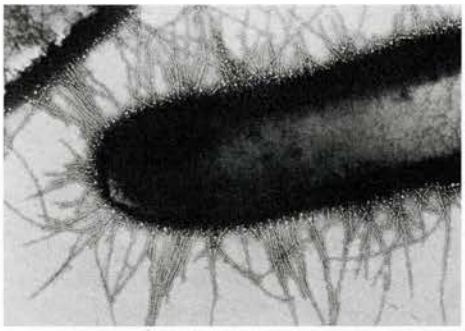
Using a technique that permits rapid searches for specific stretches of DNA, the researchers have begun correlating the expression of the different proteins responsible for binding the bacteria to human tissues—a prerequisite for infection—with switches in the basic structure of *E. coli* chromosomes.

The *E. coli* strains that interest Goldstein's group are densely covered with fiber-like surface appendages called pili. These allow the bacteria to bind to the epithelial cells lining the urinary tract. In the absence of such pili "adhesins," the bacteria cannot bind to the epithelial cells, and thus cannot cause infection.

Because the pili fibers are the major outer surface component of such bacteria, they are a major target of the human immune system. Genetic-mediated variation in the protein subunits of pili fibers enables piliated bacteria to circumvent immune system assaults, explained Goldstein.

According to the researcher, nine genes are required for the production of the pili fibers. Six or seven of these genes code for structural proteins, while the remainder play regulatory roles.

Goldstein and his associates are particularly interested in the pili tips, which appear to effect binding to the host cell. One intriguing feature of the proteins that make up the tip is that they seem to elicit only a minor immune response by infected hosts. That characteristic, noted Goldstein, may be one of the factors that has impeded efforts to create vaccines against piliated strains of *E. coli*. But the genetic-mediated variability in the makeup of these and other proteins also is an important factor.



Electron micrograph of E. coli shows numerous spiky pili that enable the bacteria to adhere to surface cells of the urinary tract. Variations in the molecular structure of pili result in bacterial strains with widely varying binding capacities and ability to trigger immune responses. (Photo courtesy of Richard Goldstein, Ph.D.)

By analyzing the structure and location of genes, the investigators have discovered a novel arrangement that may explain why some piliated *E. coli* are more virulent than others of the same strain.

The nine "basic" genes required for the assembly of a pilus are located adjacent to one another on the single, circular *E. coli* chromosome. In addition, however, the group has discovered variants of several of these genes in different parts of the chromosome. These variants, called "cassettes," can differ widely from one *E. coli* isolate to another with respect to both chromosomal location and prevalence. The differences may even be found in bacteria isolated from patients affected by identical infections.

"Our hypothesis is that when one of these variant genes is located apart from that basic segment of nine genes, it won't be expressed," said Goldstein.

On the other hand, if a similar gene cassette is occupying the key locale among the group of essential pilus genes, that genetic cassette will be expressed, and its protein will form either the tip or the major portion of the pilus. The reason, said Goldstein, is that the stretch of DNA that triggers expression of the various pilus genes appears at only one or two distinct sites on the chromosome.

Since most *E. coli* isolates were found to have several genes that potentially can encode tip or major subunit proteins, a change in gene location can lead to a dramatic change in the fibers' molecular structure, binding specificity and ability to trigger an immune response.

Neither the switching of locations by variant genes nor the larger rearrangements of whole segments of the bacterial chromosome occur simultaneously within a given bacterial population. Instead, these changes appear as a random process over time. "Most changes, in fact, probably cause the bacterium affected to become less rather than more pathogenic," said Goldstein.

He noted, however, that his group is not concerned with the occasional changes affecting a single bacterium. Their concern is the occurrence of such changes within large populations of bacteria, such as the estimated one billion *E. coli* occupying the average person's intestines. With such vast numbers, a change that seems rare becomes a fairly high-probability event.

"Our studies of *E. coli* chromosome structures," said Goldstein, "suggest there is a low but steady-state flux that leads to genetic rearrangements as successive generations of the bacteria emerge." If there is a "selective" pressure favoring a change—such as a human epithelial cell to which the bacteria can adhere and colonize because of a genetic-mediated change of its outer surface—it appears that this particular pathogenic variation, which is just one of many possible,

continued on page 6

Heart failure...
continued from page 1

and studying the effects of various drug therapies on relaxation.

"Drug therapy for heart failure previously was aimed at improving the systolic (contractile) function of the heart without recognizing that this was not the fundamental abnormality in many patients," said Apstein. "In many patients with symptoms of heart failure, the heart ejects blood normally but the relaxation (diastolic) phase is abnormal."

The Cardiac Muscle Research Laboratory group was the first to reproduce in isolated animal hearts the relaxation abnormalities that can occur in patients with stress-induced angina. They also showed that when the oxygen supply to isolated animal hearts is reduced as a result of oxygen deprivation (hypoxia) or reduced blood flow to the heart (ischemia)—mimicking conditions of heart disease or conditions that can occur during cardiac surgery, for example— the isolated hearts developed the same impairment of relaxation as seen in some patients with heart failure. The group also demonstrated that hearts from animals with high blood pressure and left ventricular hypertrophy (an enlargement of the heart) had an exaggerated impairment of relaxation during conditions of oxygen deprivation.

"In other words, normal hearts show some impairment of relaxation under conditions of oxygen deprivation, but nowhere near the extent of those from animals with hypertrophy resulting from high blood pressure. These findings fit the clinical picture that patients with myocardial relaxation problems frequently have a history of hypertension," explained Apstein.

Apstein and co-workers in the Cardiovascular Divisions at Beth Israel and Brigham & Women's hospitals in Boston, recently received a $3.8-million progam project grant from the National Institutes of Health to study diastolic relaxation in myocardial ischemia, hypertrophy and failure. Under this grant the researchers can use isolated hearts as models for patients with coronary disease or lung disease by altering the experimental conditions. For example, patients with coronary disease frequently have unstable angina—recurring periods of chest pain that result from a periodic reduction in blood supply to the heart due to coronary spasm or intermittent blood clot formation. By using isolated heart models, Apstein and his colleagues have shown that these episodes of ischemia greatly reduce the ability of the heart to relax normally. By turning coronary flow on and off in the isolated heart models, the degree of relaxation impairment and the ability of different drugs to improve or worsen the condition can be measured.

Apstein believes a major determinant of myocardial relaxation is the level of calcium inside individual heart cells, and that the reason different drugs have varying effects on heart muscle relaxation is due to the different ways in which they affect intracellular calcium regulation. If there is an impairment in the cells' mechanism for getting rid of calcium, too much calcium builds up inside cells and the heart muscle fibers cannot relax normally.

The investigators are studying beta blockers, calcium channel blockers, digitalis-type drugs, phosphodiesterase inhibiters, and glucose and insulin—all of which may affect intracellular calcium regulation differently and, therefore, can be expected to have different effects on relaxation. So far they have observed that digitalis, which commonly is used to treat heart failure resulting from poor muscle contractility, appears to make the relaxation process worse during periods of ischemia or hypoxia.

"This emphasizes the importance of distinguishing in a given patient whether his heart failure is due to a systolic or a diastolic dysfunction. If not correctly characterized, the choice of drug treatment not only might not help the patient, it might hurt him," said Apstein.

Carl S. Apstein, M.D., describes research on myocardial relaxation and related drug therapies. (Photo by Bradford F. Herzog)

In contrast to digitalis-type compounds, isoproterenol, one of a class of drugs known as beta andrenergic agonists that also are frequently used to treat patients with heart failure, did not appear to have a negative effect on relaxation when used in low doses. In addition, the group has studied the effect of high glucose and insulin on the relaxation abilities of hearts from animals with hypertension and hypertrophy and found that, during periods of oxygen deprivation, the glucose and insulin improved the exaggerated impairment of relaxation in the hypertrophied hearts and made them behave like normal hearts.

The studies of myocardial relaxation and infarct healing are being done in collaboration with W. Mark Vogel, Ph.D., associate director of the Car-

Research in Progress
Boston University School of Medicine
Office of Publication Services
80 East Concord St.
Boston, MA 02118

Non profit Org.
U.S. Postage
PAID
Boston, Mass.
Permit No. 53312

diac Muscle Research Laboratory and an associate professor of pharmacology. The program project grant research on myocardial relaxation is being carried out in collaboration with William Grossman, M.D., chief of cardiology at Beth Israel Hospital; Beverly H. Lorell, M.D., and Michael J. Cunningham, M.D., of Beth Israel Hospital; and Joanne S. Ingwall, Ph.D., and Frederick J. Schoen, M.D., Ph.D., of Brigham & Women's Hospital. Studies on hypertension and left ventricular hypertrophy are being done in collaboration with Aram Chobanian, M.D., director of the Cardiovascular Institute at BUSM and dean of the School.

—Caroline H. Lupfer

Suggested Further Readings

1. Apstein, C.S. et al: Determinants of a protective effect of glucose and insulin on the ischemic myocardium. *Circ Res. 52:* 515-526, 1983.
2. Lorell, B.H. et al: The influence of pressure overload left ventricular hypertrophy on diastolic properties during hypoxia in isovolumically contracting rat hearts. *Circ Res. 58:* 653-663, 1986.
3. Isoyama, S. et al: Acute decrease in left ventricular diastolic chamber distensibility during simulated angina in isolated hearts. *Circ Res. 61:* 925-933, 1987.
4. Wexler, L.F. et al: Enhanced sensitivity to hypoxia-induced diastolic dysfunction in pressure-overload left ventricular hypertrophy in the rat: Role of high-energy phosphate depletion. *Circ Res. 62:* 766-775, 1988.

Bacterial infection...

continued from page 4

will be propagated during a given infection. If this pathogenic variant of the organism confronts an immune system primed with a vaccine against this specific type, it will only be a matter of time until a new, nonsusceptible pathogenic variant emerges and is selected for.

Goldstein's chief aim now is to explore patterns of variability in the genes that code for adhesin pilus proteins. Despite the high degree of variability his group has discovered, he is convinced there is an underlying order to the seemingly chaotic changes affecting those genes. Creating a comprehensive picture of the possible diversity in pilus adhesin structures will permit appropriate strategies for vaccine development, he said.

Others involved in the research group include Michel Arthur, Ph.D., a BUSM National Kidney Foundation fellow; Robert Arbeit, M.D., an associate professor of medicine; Suzanne Steinbach, M.D., an assistant professor of pediatrics; Helen Crowe, M.D., an assistant professor of medicine; Lee Makowski, Ph.D., a professor of physics at Boston University; Craig Campanelli, a BUSM student; Roberta Dunn, a graduate student in the BUSM School of Public Health; and research technicians Cheung Kim and Manju Agarwal.

Goldstein's work is supported by the National Science Foundation, the National Kidney Foundation, the Fonds National Suisse pour la Recherche Scientifique, the Hood Foundation, and the Community Technology Transfer Foundation of Boston University.

—Richard P. Anthony

Suggested Further Readings

1. Arthur, M. et al: Molecular epidemiology of adhesin and hemolysin virulence factors among uropathogenic *Escherichia coli. Infection and Immunology,* in press (1988).
2. Arthur, M. et al: Structure and copy number gene clusters related to the pap P adhesin operon of uropathogenic *Escherichia coli. Infection and Immunology,* in press (1988).

Research in Progress is published by Boston University School of Medicine, 80 East Concord St., Boston, MA 01118, to communicate the excitement of current research at the School of Medicine and the School's concern for improved health care in contemporary society.
Research in Progress is produced by Boston University Medical Center's Office of Publication Services, Owen J. McNamara, director, Marjorie H. Dwyer, associate director. Cynthia A. Koury is editor. Catherine M. LeBlanc is staff artist. Donald R. Giller is director of Marketing and Public Affairs. Inquiries regarding research may be made to Daniel S. Bernstein, M.D., director of the Office of Industrial Liaison, at (617) 638-4575.