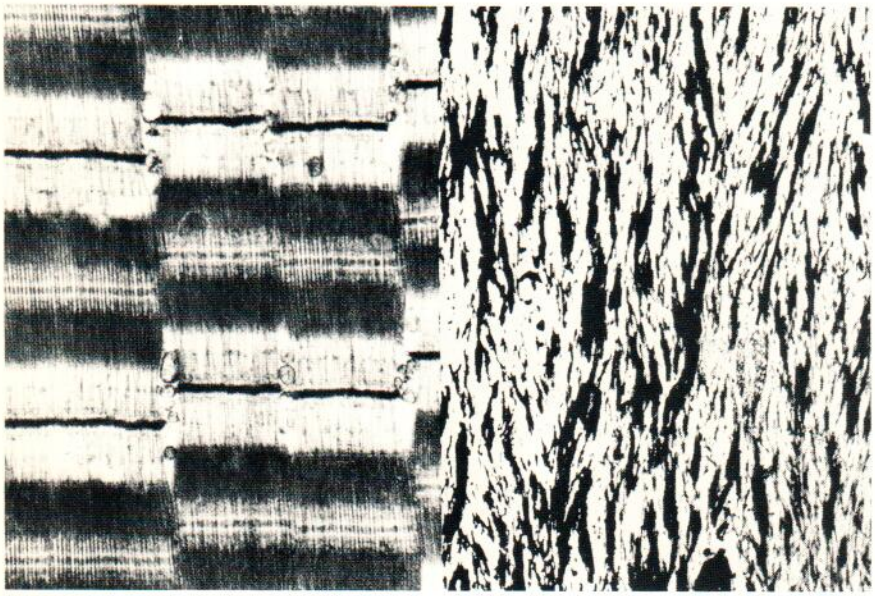
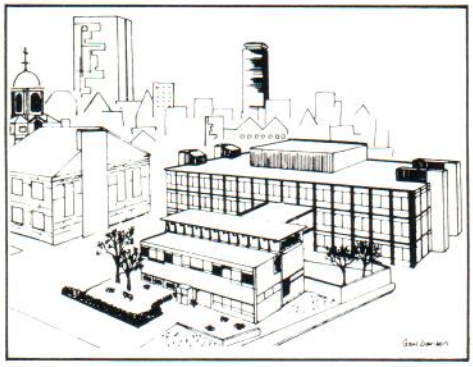
Boston Biomedical Research Institute

1991 Annual Report

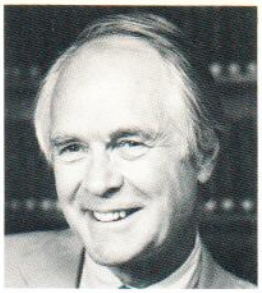


BOSTON BIOMEDICAL RESEARCH INSTITUTE

is an independent, non-profit organization with a staff of M.D. and Ph.D. investigators who carry out a broad program of basic research in biology and medicine, and provide highly specialized training for future physicians and scientists. For more than two decades the Institute has maintained its position among the leaders in the world-wide effort to prevent and cure disease. Areas currently under investigation range from the study of birth defects to the biology of aging. The findings of Institute scientists are used in clinical research aimed at helping people suffering from cancer and diseases of the heart, muscles, liver, and eye and thus the Institute's research programs contribute to the well-being of mankind.



Cover photo:
Electron micrographs of striated (at left) and smooth (at right) muscle filaments (magnification 15,000X). Note the high ordering of filaments in striated muscle compared with smooth muscle.



A significant portion of the activities of the Trustees during the year has been taken up with the search for a new department head/executive director, which I have mentioned in past reports. The search process has been thorough, involving the identification, through submitted resumes, of candidates to be interviewed both at Harvard and at the Institute. These selected candidates also presented formal seminar talks. This process resulted in identifying a number of well qualified candidates, which was most encouraging. A good deal of time is, understandably, taken up in working out the arrangements under which a leading candidate would assume the joint BBRI/Harvard Medical School position. This search is, of course, complicated by the fact that BBRI and the Harvard Medical School must not only agree on a candidate but go through their own separate appointment procedures. This process is ongoing as this report is written; thus nothing more definitive can be stated at this time. It can be stated, however, that the search itself has generated a number of worthwhile discussions between the candidates and representatives of the Institute's staff and Trustees as to the strengths and needs of BBRI, which have been stimulating and beneficial. The search process itself has helped us to sharpen and focus our analysis of the Institute's future direction.

A related effort in the past months has been to begin preparations for a capital fund drive for the Institute. It became clear in discussions with many of the candidates that the size of the Institute's endowment is too small to support the ambitious and expanded research programs which are implicit in the hiring of a senior scientist who would head up a new research department, as well as act as executive director. To attract the qualified scientists to staff a new department (in addition to its director) will require endowment funds to assure candidates of funding for their salaries during a transition period while NIH grants are obtained. Furthermore, as John Gergely's report indicates and has been stated in previous reports, the more stringent NIH procedures require the Institute to be more flexible in providing short term support for investigators who are delayed in obtaining or renewing grants. The combination of these needs has led the Trustees to the decision that we should seek to build up our endowment funds by way of a capital campaign, and efforts to this end have been initiated. Crucial to such a campaign would be the appointment of a new executive director and the articulation of his or her vision for the Institute. Thus we will await the resolution of the search before formally embarking on the campaign.

During the year, Dr. Peter Davison, the long time director of the Department of Fine Structure Research, resigned this position. He has served the Institute with competence, good humor and wise counsel for 22 years both as a scientist and periodically as Executive Director. He continues as a Senior Scientist working on research projects in which he has a particular interest. The Trustees honored Peter at a dinner earlier this year, at which he left us with some provocative thoughts on the nature of scientific research.

Also, I want to make note of the talented and hard working scientific staff at the Institute who continue to bring credit to themselves and BBRI with their ongoing research. It is particularly noteworthy that this work is done so well despite the distractions, which must be significant, caused by the constant necessity to compete for research grants in an increasingly competitive and uncertain funding climate.

The donors to the Institute are acknowledged in this Annual Report, but I would be remiss if I did not call attention to them here. As has been said before, the annual contributions to the Institute assume greater importance during these times of tighter money from the National Institutes of Health. We are most grateful for the support of all of our donors, new and old. As this report goes to press, we have just received word of a grant of $300,000 from the Amelia Peabody Charitable Fund for addition to our endowment to provide assured support for a senior scientist. This magnificent gift is a most welcome vote of confidence in BBRI's mission and its future.

John B. French

As we look back on the past year it seems that, compared with the revolutionary events that have taken place in the world at large, life has been pretty quiet at BBRI. Nevertheless, a number of things have happened or are in progress that are bound to have important consequences for the future of the Institute. The search set in motion about a year ago for a joint BBRI Director/HMS Professor appointment has led to the identification of a number of highly qualified candidates but, as is not unusual with academic searches, the time needed for completing the process is longer than hoped for at the beginning. An exciting aspect of this effort headed by Drs. Elkan Blout and Elizabeth Hay is the prospect of closer ties with Harvard Medical School that might open up new opportunities for research collaboration and participation in teaching by BBRI faculty.

As before, we have been concerned throughout the year with the reduction in the percentage of grants funded at NIH, the main funding source of the Institute. It was, therefore, particularly heartening that one of our investigators, Terry Tao, has sailed through his competitive renewal with flying colors, receiving not only prompt renewal but also a MERIT award with a term of at least eight, possibly ten, years. These awards are made to a rather small number of investigators with an established record of outstanding achievement. Another investigator, Renne Lu, although in the "outstanding" category, had to wait more than a year before receiving the welcome news of a competitive award. Although I cannot put an accurate number to my evaluation, it seems to me from discussions with colleagues at various meetings that BBRI is still doing better than average compared with a number of outstanding institutions in competing for NIH grants.

The scientific activities of BBRI are reflected in the papers published during the past year in high quality journals with a strict system of peer review by expert referees. (Three of the Institute's investigators are members of such review boards.) What is even more important, BBRI scientists are not only developing themes started many years ago, but are branching out into areas which they have started cultivating in the last few years and whose blossoming into full flower is likely to occur in the future. In this vein an application for a major research grant focussed on smooth muscle, a topic described in more detail in this annual report, has been submitted by a group of investigators in the Department of Muscle Research. The extension of current work on striated muscle to smooth muscle, and possible future work on other motile systems in non-muscle cells, will provide handsome payoffs on the effort invested by BBRI investigators in research in related areas during the last three decades. With this type of program expansion, together with the appointment of a new senior investigator to establish a new program in the area of cell biology, particularly dealing with the molecular aspects of developmental biology, BBRI can look forward to many successful years ahead.

Although NIH is going to continue to be the main support agency for BBRI, it is clear that increased contributions from the private sector are sorely needed. Even if NIH funding improves in the next few years, current policies have decreed that cuts made during the preceding years be frozen, in the sense that future increases in funding will remain tied to current reduced levels. To put it differently, NIH funds are increasingly inadequate to fully support the work for which the award was made, particularly with respect to the recruitment of junior associates whose ideas and youthful energy are an essential mix for a successful research program. While the Trustees have in the past made it possible to use reserve funds for bridging gaps in funding, it is also clear that our staff perceives a lack of stability owing to our inability to guarantee salary support, even to those with tenure. This was the case recently when one of our senior scientists, Dr. Tai, decided to accept a position with guaranteed institutional funding and, what is even more important, with institutional funds for fellows and trainees. The funding of training programs is an area that, I believe, will have to be addressed, so that BBRI faculty will be able to bring on board promising young colleagues who then would be able to acquire funds of their own. At present our inability to make offers to promising candidates prior to obtaining training funds usually results in losing these prospects.

I should like to close by expressing the Faculty's thanks to the Trustees and Corporation whose support, not only in material terms but also through their advice and encouragement and by their acting as ambassadors to the community at large, has been a key element in making BBRI the outstanding research institute it is now.

John Gergely, M.D., Ph.D.

SMOOTH MUSCLES— THE HIDDEN MOVERS

WHAT IS SMOOTH MUSCLE?

When the word muscle comes up, most people would immediately think of physical activity, sports, ballet, the skillful movement of an artist's hand, the surgeon's hands carrying out a delicate operation. They would also think of the heart, the muscular pump which delivers blood to every cell of the body day in and day out. The muscles that are involved in all these activities are referred to as striated muscles because of their appearance under the microscope.

Probably fewer people would think of activities associated with what are known as smooth muscles—yet these muscles play an equally if not more important role in the life of the organism. This lack of awareness is because smooth muscles are hidden from our eyes and are therefore easily forgotten or not even known to many of us. No one can display a smooth muscle the way body builders can display a biceps or any other bulging muscles on their bodies. Where, then, are the smooth muscles, and what do they do?

Hardly any organs of our bodies can function without smooth muscles. Smooth muscles are an important component of the walls of blood vessels and serve to control their diameter. When these muscles do not perform their job with perfection, well-known ailments such as high blood pressure or coronary heart disease occur. The whole process of the movement of food through our gastrointestinal system depends on smooth muscle, as does the delivery of bile—an important ingredient in processing our food—which is transported from the

Albert Wang discussing results of gel electrophoresis with Shuang Xu.

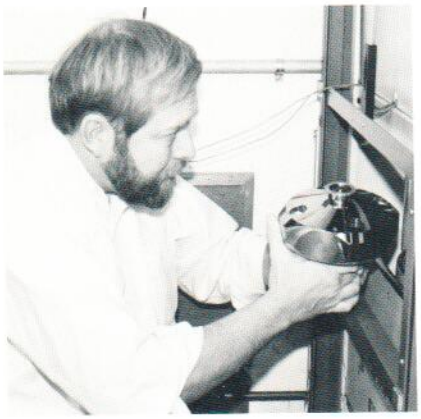
liver through ducts whose walls are under the control of smooth muscle.

The volume of our airways is controlled by smooth muscles, and diseases such as asthma arise from malfunction of these structures. Reproduction of the human—and for that matter of any other animal—heavily depends on smooth muscle. Childbirth involves an amazing growth of a smooth muscle organ, the uterus, whose powerful contraction leads to delivery of the newborn. Even the sperm, which has its own motor, has to be delivered with the assistance of various smooth-muscle-powered systems to achieve fertilization.

WHAT MAKES SMOOTH MUSCLE CONTRACT?

It is perhaps surprising that despite the importance of smooth muscle in health and disease relatively little is known about many details of its operation compared with striated, or voluntary, muscle. The basic mechanism of contraction in smooth muscle is essentially the same as in striated muscle: two sets of filaments made up of two distinct proteins, myosin and actin, slide past each other, developing tension and leading to a wave of contraction propelling the contents of those ducts in whose walls smooth muscle occurs. Skeletal muscles are under voluntary control, and signals for contraction are initiated by an impulse from our brain reaching the muscles through their nerves. Smooth muscles, on the other hand, are under the influence of a variety of factors including the so-called autonomous nervous system. Most of us are unaware of this subset of the nervous system, which operates without our being conscious of it and delivers both stimulating and inhibitory signals. In addition a wide variety of hormones and other chemical substances generated in various parts of the body may also initiate or modulate the contraction of smooth muscle.

The immediate signal for smooth muscle contraction is the release of calcium ions from cellular storage sites. In this respect smooth muscles appear similar in their control mechanism to striated muscles. But in striated muscle, calcium combines with a constituent of the so-called thin filament which then triggers the interaction of the two kinds of proteins making up the thick and thin filaments. In smooth muscle, on the other hand, a more complex reaction is initiated by calcium, leading to the attachment of a phosphate group to the motor protein—that is, to myosin—itself. In recent years results obtained in various laboratories have suggested that, in addition to this distinctive mechanism, another mechanism of control may operate in smooth muscle, in a manner very similar to that found in striated muscle; the elucidation of this second control system has just begun.



Walter Stafford loading an analytical rotor containing a sample of muscle protein into the ultracentrifuge.

At BBRI, interest in smooth muscle goes back more than ten years, research in this field having been initiated by the late Jack Seidel. After Jack's death, Albert Wang continued research along these lines with financial support from NIH, the American Heart Association, and the General Cinema Corporation. The time now seems ready for mounting a major program with the participation of a number of staff members of the Department of Muscle Research. To capitalize on both earlier and ongoing work at BBRI, plans are now under way for the submission of a major grant application, involving collaboration with a group of colleagues in Texas, under the "program project" grant system of the NIH. If the application is successful, the five-year grant would amount to over $1M annually. This report presents some of the background and outlines our plans for this major effort.

The contractile machinery of smooth muscle

It is useful to view the specific problems relating to the contraction of smooth muscle from the broader perspective of biological movement and force generation. Modern cell biology has uncovered a number of motile force-generating systems in addition to the more familiar contractile systems long studied in muscle. All motile systems—that is, systems in which something moves—can be thought of in terms of a force-generating motor, tracks on which the motor moves, control systems that regulate movement and force, and fuel used for performing work. But unlike a railway, the motor and the tracks are made not from steel but from protein and have molecular dimensions.

The smooth muscle system resembles in many respects the machinery found in striated muscle. In both striated and smooth muscle the energy is provided by a molecule known as ATP, which is generated from the food we ingest by a series of chemical transformations. In both types of muscles the motor is a complex protein molecule named myosin. Each myosin molecule consists of three pairs of distinct components: one pair of heavy chains and two pairs of light chains. The myosin motor runs on a track made up chiefly of actin and a few other accessory proteins. The regulation of its movement depends in both systems on calcium ions whose level increases when the nerve stimulus reaches the muscle cell.

On the other hand, there are fundamental differences between smooth and striated muscle with respect to the mechanisms of the regulation of contraction. Calcium ions combine with different molecules—so called receptors—in smooth and striated muscle, and the result of the different combinations is also different. A characteristic feature of smooth muscle is that calcium initiates a chemical change, catalyzed by an enzyme, which results in the formation of a bond between phosphate and myosin, a process called phosphorylation. An important aspect of current and future research relates to the question of how changes in myosin brought about by phosphorylation can lead to muscle contraction.

Renne Lu operating the automatic protein sequencer.

How do changes in the shape of myosin molecules cause contraction?

To address this question, one first has to focus on changes that occur in the shape of the myosin molecule as a result of phosphorylation. Scientists like to think in terms of models that guide them in figuring out what really takes place at the molecular level. The structure and shape of the myosin molecule suggest a model for the interaction of the myosin motor with actin—the chief constituent of the track—that can lead to tension or motion. According to this model, the long tails of the myosin molecule combine into a cable-like structure, while the so-called heads of the myosin molecules—there are two per molecule—protrude along the surface of the cable and are capable of attaching to actin. The junction of the heads with the rest of the myosin molecule appears to be flexible, and it is possible that motion of myosin along the actin fibers involves a hinge-like action at the junction. Indeed there is evidence that the breakdown of the fuel molecule ATP causes a change in the orientation of the heads. As a result, the myosin heads carry out oar-like motions which propel the myosin filament along actin, resulting in movement or, if movement is resisted, in force.

Zenon Grabarek discussing experimental results with Yasuko Mabuchi.

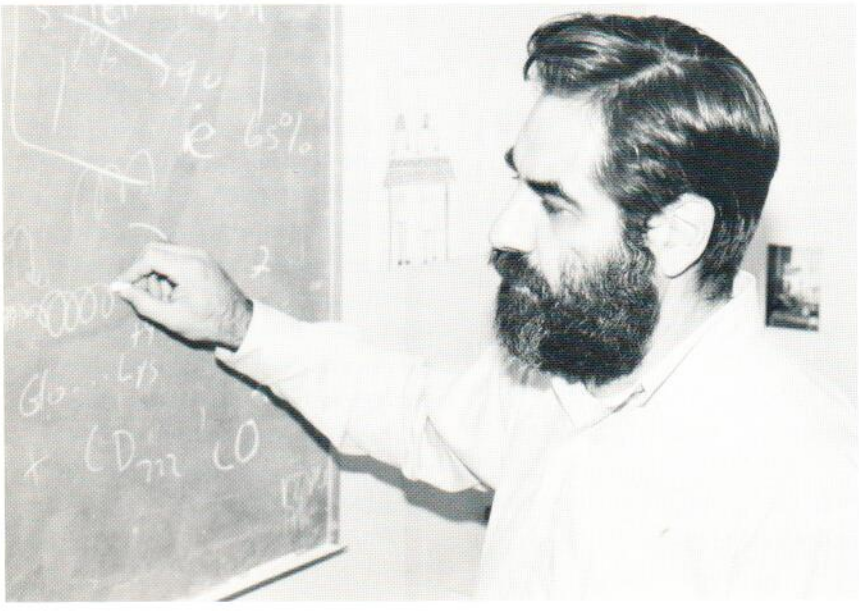
Recent evidence suggests that this model may be over-simplified and that subtle changes also occur within the head itself. Thus it is important to explore changes in the proteins which constitute the head portion of smooth muscle myosin that accompany the contractile process. A number of techniques are available for exploring changes in the shape of a protein. Among these, the so-called cross-linking techniques play an important role, because they permit the identification of regions in a protein that are close enough so that a chemical bond can be formed between them. Subsequently the protein can be cut into small pieces, and the chemical identity of the pieces joined by the cross-link can be determined. If different portions of the myosin molecule come within striking distance of each other when the muscle exerts force, this would indicate that movement has taken place within the protein, and one would gain insights into the molecular mechanisms that generate force. Other techniques to probe molecular changes during muscle activity involve the attachment of small molecules—probes—at suitable positions within myosin. These probes are able to absorb energy from light and transmit it to another probe attached to another portion of the protein. The amount of light energy that can be so captured can be used to determine the distance between different points of the molecule, and this again will open up ways to detect changes that underlie force development. Studies of this nature have been carried out in great detail in striated muscle, but little information is available on the motor of the smooth muscle. Modern techniques based on genetic engineering are very helpful in carrying out measurements of this kind of energy transfer because they make it possible to introduce components into the molecule that can serve as sites for the attachment of light-absorbing and light-emitting molecules.

How is the contraction of smooth muscle activated?

Another important area of study is the very process that leads to activation of the contractile machinery of smooth muscle in response to changes in the concentration of calcium ions. This is the process of phosphorylation catalyzed by an enzyme called myosin light chain kinase, which is known to scientists by the acronym MLCK. This enzyme itself does not respond directly to calcium, the signal substance indicated earlier; rather, its activity is modulated by combining with another protein, known as calmodulin, which is the actual calcium binder (or calcium receptor). Calmodulin shares many structural properties with a protein that plays a crucial role in the regulation of striated muscle, namely troponin C. Experience gained in our earlier studies on troponin C (see last year's Annual Report) promises to be helpful in the modification of calmodulin by genetic engineering. MLCK works as a switch when calmodulin plus calcium combine with it. As the switch opens, a region of the molecule becomes available for reaction with the myosin component to be phosphorylated. It is important to find out as much as possible about the details of the normal phosphorylation of smooth muscle myosin. The knowledge thus gained will serve as a blueprint for understanding the pathological changes in diseases in which any of the components participating in the complicated reaction of calcium-controlled phosphorylation may be defective.

Defective molecules have been found in an increasing number of diseases—e.g. muscular dystrophy, cystic fibrosis—and the identification of the defective component opens the way for using the techniques of genetic engineering to restore normal function.

In recent years, evidence has been accumulating that one or more other systems of regulation may exist in addition to the process of myosin phosphorylation, which is the hallmark of regulation of smooth muscle contraction. Two fairly new proteins, caldesmon and calponin, share the limelight with an old friend of muscle scientists, tropomyosin, which is part of the regulatory system in striated muscle. Caldesmon is a rather large protein that binds to the actin filaments, although its precise mode of binding still needs to be defined. Many details of the molecular structure of caldesmon have

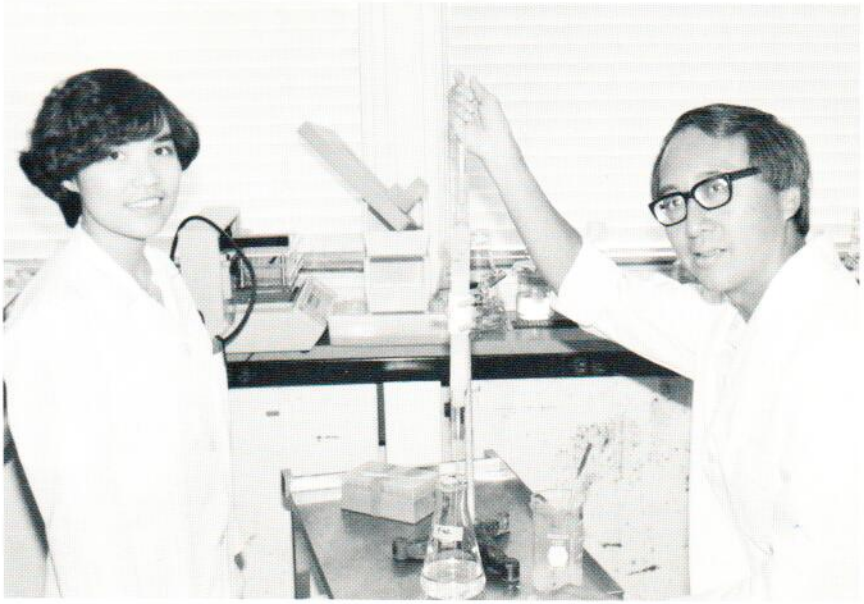


Philip Graceffa speculates on the structure of caldesmon, a vascular smooth muscle protein which may play a role in hypertension.

been elucidated, and portions of the molecule have been identified as distinct binding sites for actin and for myosin. Techniques of genetic engineering have made it possible to generate fragments containing binding sites for these proteins, and it may be possible to identify their precise functional role by using antibodies directed against specific regions of caldesmon. Caldesmon inhibits the activity of the smooth muscle system even when it is "turned on" by the phosphorylation of myosin. The effect of inhibition by caldesmon is reversed by calcium which, as is the case for activation of phosphorylation, also requires the intermediary calmodulin.

The newest protein implicated in regulation via the actin-containing filament is calponin. Interestingly, one of the discoverers of calponin, Katsuhito Takahashi, for the past two years a post-doctoral fellow at the Childrens Hospital of Boston under Dr. Nadal-Ginard, participated in the Symposium dedicated to the memory of Jack Seidel, and this led to a collaborative project with BBRI scientists. The molecular cloning of the calponin gene and the study of its expression promise interesting developments leading to a better understanding of its function. Many questions including the role of calcium or of phosphorylation in the regulation of calponin activity await clarification.

Sam Lehrer considering results of dichroism experiments on muscle fibrils.



Terry Tao running an ion exchange column with Hongmei Lee.

Much work remains to be done on the role of tropomyosin in the regulation of smooth muscle activity. It is yet to be established whether tropomyosin plays merely a structural role, for example by enhancing the rigidity of the thin filaments, or whether it is indeed part of the switching mechanism in striated muscle in which the thin filament changes from an "off" to an "on" state. An important feature in striated muscle is the ability of tropomyosin molecules to form connections with their neighbors along the actin filament, thereby permitting the binding of a myosin molecule at one point along the filament to increase the activity of the filament some distance away. It remains to be established whether this so-called cooperativity plays a role in the regulation of smooth muscle and how the various regulatory systems, one acting on myosin and the other on the actin filament, interact with each other. A good way to monitor all the complex systems is to measure in test tube experiments the rate at which reconstituted actin-myosin-tropomyosin systems containing various other regulatory components bring about the breakdown of ATP, thereby simulating the flow of energy in the actual living cell. Another important tool will be to utilize actin filaments labelled with fluorescent markers and follow the movement resulting from the interaction with myosin and ATP by direct monitoring of the microscopic image with a video camera.

Katsuhide Mabuchi examining a smooth muscle protein under the electron microscope.

The challenge

The complexity of the smooth muscle field may appear discouraging. Yet it is a challenge to scientists, and it is hoped that as some of the questions outlined above receive answers through the use of state-of-the-art techniques of physical biochemistry, immunochemistry and genetic engineering, new hope will be kindled for the eventual eradication of a wide spectrum of diseases.

An important part of research is the communication of scientific discovery so that the knowledge gained can help new research as well as benefit clinical studies directed towards curing or preventing disease. The dissemination of new research findings is achieved primarily by publication in scientific journals. Over the past year, BBRI investigators have published the following papers:

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Kantham, L., R. Raychowdhury, K. Ogata, A.A. Javed, J.R. Rice & D.R. Sanadi, 1990. Amino-terminal amino acid sequence of beef heart mitochondrial coupling factor B. FEBS Lett. 277:105-108.

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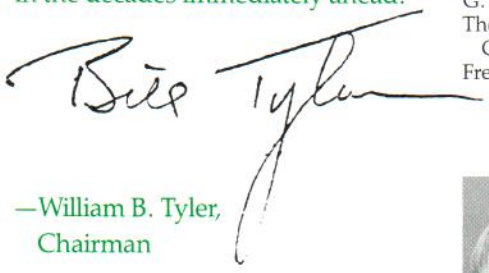
—*to the many friends* whose gifts to the Annual Research Fund totalled $114,000 and helped some of our scientists to bridge NIH funding gaps that are being experienced in biomedical research laboratories nationwide. Both bridging support and seed money for testing innovative research concepts are ongoing needs which rely on the Annual Research Fund and are critical to the continued success of our investigators.

—*and to the several friends* who made capital gifts—in the amount of $78,600 together with pledges for a further $225,000 payable over the next several years—to address needs pivotal to the Institute's future: development of a new research department which will draw on present strengths of BBRI and shift the focus of our research to meet priority biomedical challenges in the decades immediately ahead.



—William B. Tyler,
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Dr. Mahlon Hoagland, after-dinner speaker at the Annual Meeting, talking with BBRI Vice President Dr. Elkan Blout.

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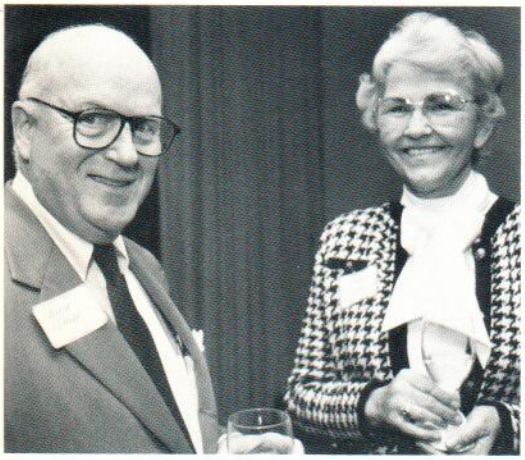
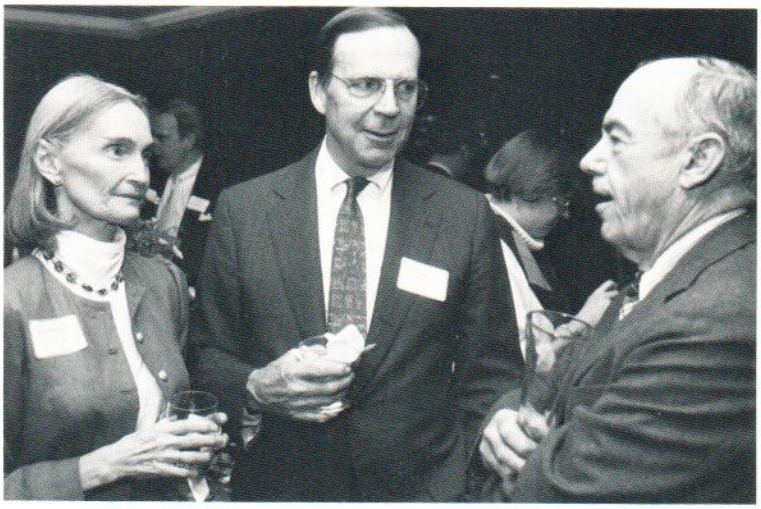
Corporation member Fred Corneel (center) with wife, Marty, and BBRI Treasurer Ernie Henderson.



Richard Leahy, guest, with Vice President Anne Stone.



Corporation member Barbara Leith with her husband, Bill (center), and guest Nelson Darling.

We gratefully acknowledge the individuals who have given to Boston Biomedical Research Institute so much of their time, energy, and expertise.

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A sub-committee of the Board meets to discuss recruitment of a director. (Left to right) Henry Paulus, Elkan Blout, Ernest Henderson, John Shane, David Gibbs, and John French.

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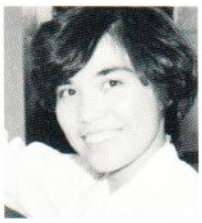

Hongmei Lee

Zenon Grabarek

BOSTON BIOMEDICAL RESEARCH INSTITUTE
BALANCE SHEETS
AUGUST 31, 1991 AND 1990

	1991	1990
ASSETS		
CURRENT ASSETS		
Cash	$ 1,231,708	$ 1,581,156
Grants receivable	2,871,111	3,445,321
Pledges receivable	—	960
Prepayments, deposits and other receivables	166,434	148,597
Investments, at market value (cost 1991—$3,998,755 1990—$3,542,886)	4,748,327	3,929,465
Total current assets	9,017,580	9,105,499
FIXED ASSETS		
Leasehold improvements	1,935,632	1,935,632
Research equipment	4,782,484	4,630,268
Total	6,718,116	6,565,900
Less accumulated depreciation	5,278,790	4,958,609
Net fixed assets	1,439,326	1,607,291
	$10,456,906	$10,712,790
LIABILITIES AND FUND BALANCES		
CURRENT LIABILITIES		
Accounts payable and accrued expenses	$ 60,837	$ 180,419
Deferred grant income	3,118,424	3,767,358
Deferred fund (building)	115,702	115,702
Total current liabilities	3,294,963	4,063,479
FUND BALANCES		
Unrestricted	5,274,873	4,730,694
Restricted	447,744	311,326
Fixed assets	1,439,326	1,607,291
Total fund balances	7,161,943	6,649,311
	$10,456,906	$10,712,790

BOSTON BIOMEDICAL RESEARCH INSTITUTE
STATEMENTS OF REVENUES, EXPENSES AND CHANGES IN FUND BALANCES
FOR THE YEARS ENDED AUGUST 31, 1991 AND 1990

	1991	1990
REVENUES		
Grants	$5,123,006	$5,065,122
Equipment replacement	139,394	156,456
Contributions and pledges		
Unrestricted	113,686	143,947
Restricted availed of in current period	19,629	207,891
Property and equipment purchased	152,216	211,955
Investment income		
Interest and dividends	291,755	312,160
Realized and unrealized gains on securities	630,051	(34,810)
Total	6,469,737	6,062,721
EXPENSES (by department)		
Muscle Research	2,296,862	2,561,922
Cell and Molecular Biology	1,661,153	1,418,978
Metabolic Regulation	1,091,196	894,222
General Research	566,331	568,517
Fund Raising	65,252	51,944
Purchase of fixed assets	21,482	2,978
Depreciation	320,181	350,817
Write off-subsidiary advances	11,148	14,491
Total	6,033,605	5,863,869
EXCESS OF REVENUES OVER EXPENSES	436,132	198,852
Restricted fund contributions	76,500	—
FUND BALANCES, BEGINNING OF YEAR	6,649,311	6,450,459
FUND BALANCES, END OF YEAR	$7,161,943	$6,649,311

Copies of our complete, audited financial statements, certified by the independent accounting firm of John Vecchi, CPA, are available upon request from the Comptroller, Boston Biomedical Research Institute.

Credits

Photos:
Page 1, John Ganson; pages 2–8, 12, 13, Zenon Grabarek;
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