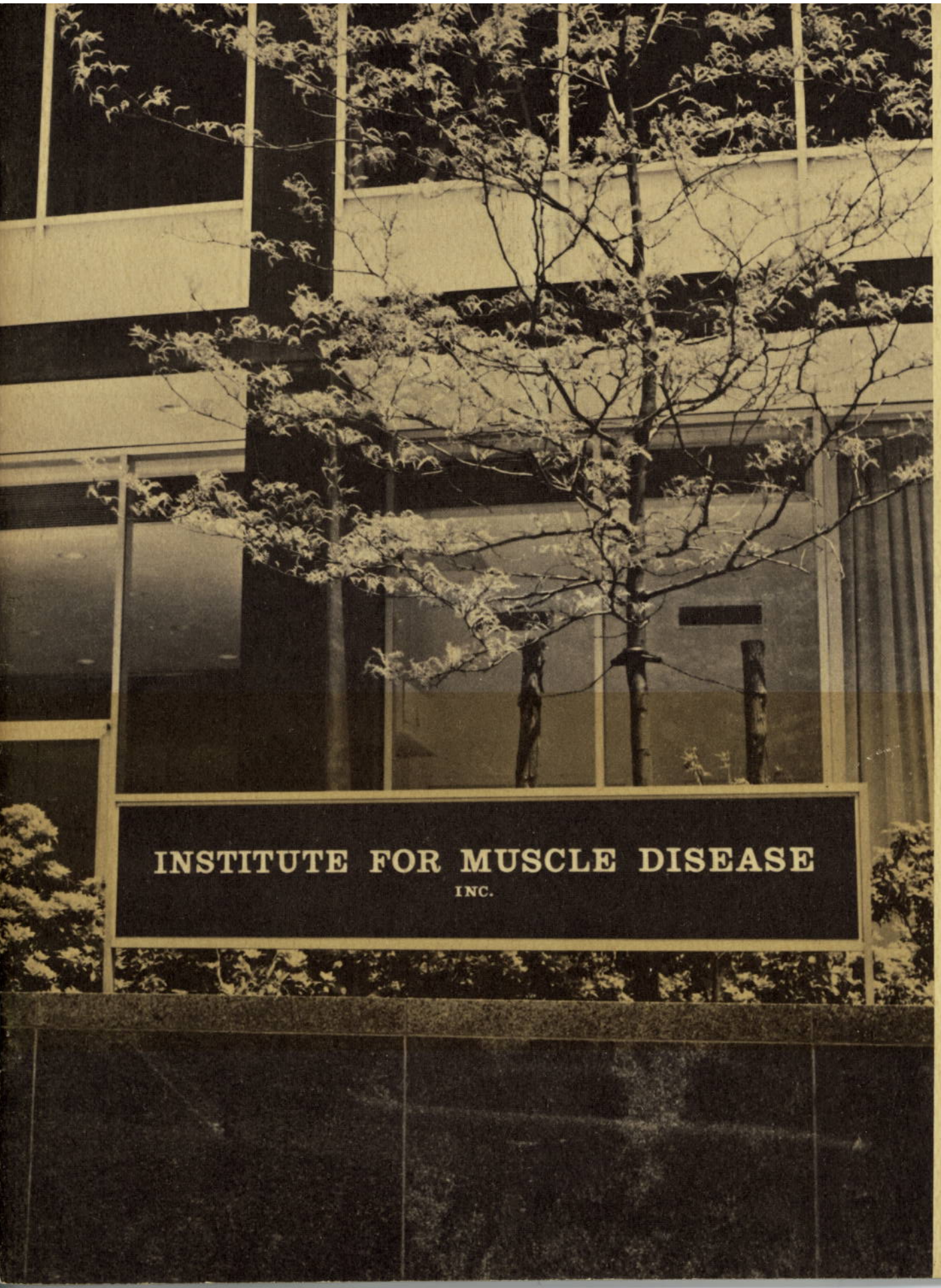
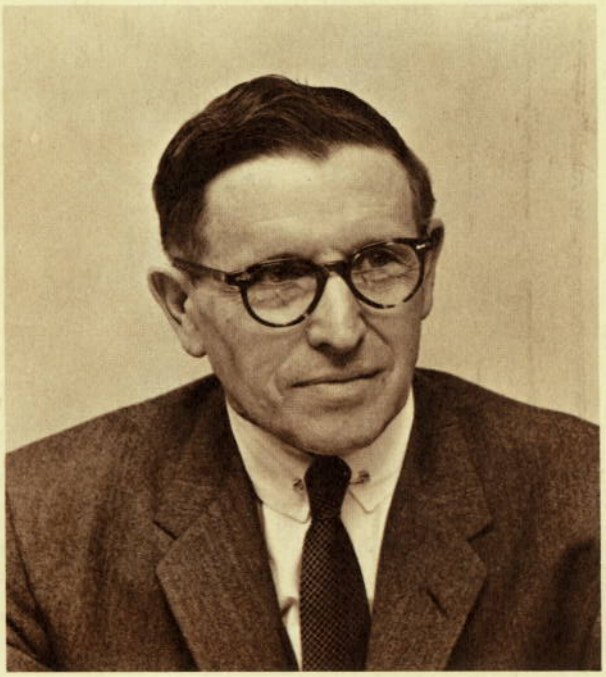

INSTITUTE FOR MUSCLE DISEASE
INC.



Dr. Ade T. Milhorat
Director, Institute for Muscle Disease, Inc.

Dr. Ade T. Milhorat studied at Columbia and Cornell Universities where he received his A.B. and M.D. degrees respectively. He received his hospital training at Presbyterian Hospital, New York City, and, as a research fellow of the National Research Council, he studied and did research in chemistry for two years in Leipzig, Germany. After his return to this country, he received appointments in the Departments of Medicine and Pharmacology, Cornell University Medical College and New York Hospital, and in the Russell Sage Institute of Pathology. Presently, he is Professor of Clinical Medicine, Cornell University Medical College , and Attending Physician, New York Hospital.

During his research in Leipzig, Dr. Milhorat came into close contact with the problem of muscular dystrophy. In 1950, he assisted in the establishment of Muscular Dystrophy Associations of America where he is Chairman of the Medical Advisory Board. He is a member of many medical and scientific societies, a past president of the New York State Board of Medical Examiners, member of the Medical Board of Muscular Dystrophy Association of Canada, honorary member of the French Association for Muscular Dystrophy, and a director of the National Hospital for Speech Disorders and of the U.S. Committee of the International Society for the Rehabilitation of the Disabled.

"Strong reasons make strong actions"

Baffling neuromuscular disorders, always crippling and often fatal, continue to cause suffering to millions throughout the world. Here is the strong reason that sparks sustained and powerful action at the Institute for Muscle Disease, a research facility in New York City which has been in operation since December, 1959. Its twin goals—more precise knowledge of muscle and its pathologies, and methods of curing or at least arresting these disorders.

In the last decade or so, the long-neglected problems of muscle disease have been receiving increasing attention, largely through the instigation of Muscular Dystrophy Associations of America (MDAA), the national voluntary health agency which was responsible for the creation of the Institute. MDAA's widely distributed grants have stimulated research in the field in many foreign countries as well as in the United States, and this in turn has generated other sources of support. The reader may ask: Is there any need then for this new muscle research center?

Indeed there is. As biological inquiry extends its frontiers, laboratory space grows tighter everywhere. A scientist who lacks up-to-date equipment for his work may succeed in acquiring it with the help of a grant; but in all probability, if he moves to another institution, he must leave the costly investment behind—a poor disposition of research funds. Moreover, it is difficult to coordinate research carried on in widely scattered places.

The Institute for Muscle Disease was conceived to surmount such limitations. It provides a number of new, well-designed laboratories. Its expensive, ultra-modern equipment is shared by a large group of scientists. Working side by side in a single institution, they can more easily avoid duplication of effort and spark each other's thinking. Out of such cross-fertilization of ideas, from workers in different disciplines, may come new leads which those on the spot can promptly reorient themselves to pursue.

As the only center anywhere devoted specifically to the study of muscle and its disorders, the Institute is likely to become a central clearing house and focal point for world-wide muscle research. It already offers invaluable experience in advanced research methods to visiting fellows, and members of its staff participate in annual training institutes for physicians in the diagnosis and management of muscle diseases. The Institute hopes eventually to establish a muscle registry—a sort of "library" of microscopic slides and micrographs, accompanied by case histories—illustrating the various forms of muscle disorders found throughout the world.

"An instrument created...

The architects have designed a handsome eleven-story building which is at once a group of laboratories and a workshop, a center of learning and a research hospital in miniature. Consulting at every step with scientists, in particular with muscle-disease research pioneer, Dr. Ade T. Milhorat, who was to become the Institute's director, they were challenged to provide a setting not only for the most advanced research equipment of the day but also for tools and techniques not yet invented.

This challenge was met in part by adopting a central tower cantilever design. From a weight-bearing core containing elevators, stairways and other special facilities, utilities are distributed to racks on each ceiling to supply the floor above. Unencumbered by pipes and power lines and bearing no weight, walls can be shifted with relatively little labor and expense—say, to accommodate an exceptionally large piece of equipment. And, since all laboratory floors follow the same basic design, it will be no great problem to switch them, if necessary, from one function to another.

Anticipating the use of more and more electronic instruments, the building is generously supplied with electrical facilities. Outlets for these, as well as for gas, water and air, are grouped in convenient service strips at bench height along the walls of the laboratories, where all plumbing is acid-resistant. Finally, an instrumentation center headed by an electronics expert stands ready to design and build wholly new equipment as the need arises.

Dr. Fritz Buchthal, Director of the Institute of Neurophysiology at the University of Copenhagen, conducts a special seminar on electromyography. Such presentations by visiting scientists are a feature of the IMD's program for interdisciplinary exchange among scientists.

The five floors of laboratories provide ample working space for fifty Ph.D.s and M.D.s (some thirty are now at work) and about one hundred technical assistants. A photography center, a medical-illustration studio, and a 10,000 volume reference library, with three sound-proofed cubicles for dictation and the reading of microfilm, assist the scientists in preparing reports, articles and lectures on their work. In the metabolism unit on the 10th floor, up to twelve research patients of both sexes and different ages can be housed at one time. Pleasant one, two and three-bedrooms with private baths offer every modern convenience to these wheelchair invalids, who receive the best of medical and nursing care during the investigations.

Animal quarters on the 3rd floor are a model of hygienic housekeeping. A special elevator conveys new arrivals straight from the service entrance to the quarantine station. Two huge washing machines—one for small cages and one for large—automatically clean and dry hundreds of animal cages in an hour. Tiled walls, flooring treated to prevent seepage, and handy hot-and-cold-water hose connections make it convenient to hose down the animal rooms routinely from top to bottom.

For staff and visitors, too, housekeeping arrangements are admirable. The attractive cafeteria, which seats about 100, can be turned into an assembly room in minutes by closing off the kitchen areas with folding doors. A public address system and a screen are recessed in the ceiling; electrical connections for a projector are in the floor. Here the scientific staff meet bi-weekly for seminars at which they exchange reports on their work. Three smaller conference rooms supplement the cafeteria space.

Incorporated in the building are the most modern safety measures. Radioactive, volatile and combustible materials are stored in a concrete-reinforced basement. Specially shielded micro-curie and milli-curie ("hot") rooms are used for research with radio-isotopes. (All staff concerned with istotopes wear badges that give warning by changing color of any sizable increase in exposure to radioactivity.) To insure that no one is accidentally locked in, incubator and cold rooms are fitted with inside door catches.

Air conditioning throughout does away with limitations on research hitherto imposed by the weather. Metabolism units have customarily closed down during the summer months, because heat and humidity distort their findings on patients. Many other scientific experiments are adversely affected by extreme changes in temperature. But here separate controls of temperature and humidity in individual laboratories, as well as incubator and cold rooms, permit uninterrupted use of the entire physical plant the year round. With much of the equipment automated to perform complex tasks unattended, the research goes on in high gear, literally day and night. Awareness that time is running out for the victims of progressive muscle-wasting adds a note of urgency to the whole program.

Strategically located close to the New York Hospital-Cornell Medical Center, the Institute has sources of fresh human tissue, needed for many research projects, practically on its doorstep. From the operating rooms of the hospitals specimens can be rushed on ice straight to the Institute laboratories.

...for a still greater creation"

Equipped with this superb instrument, the Institute's scientists may succeed in finding the life-saving key (or keys) to the riddles of muscle disease. This is the great purpose.

Among the primary diseases of muscle, *muscular dystrophy* (MD) appears in three or possibly more forms. The Duchenne or pseudohypertrophic type (PMD) strikes in childhood, mainly boys. Deposits of fat in the leg muscles at first give a false impression of sturdiness, but the wasting of muscles and their replacement by fat and connective tissue proceed rapidly, with early contractures and eventual total crippling. In the absence of any known treatment for reversing or even delaying the progress of the disease, respiratory illness, with which weakened breathing muscles cannot cope, is sooner or later fatal—usually before the patients are grown. Occasionally, involvement of the heart muscle is the precipitating cause of death. Facio-scapulo-humeral MD, so-called because it first attacks the muscles of the face, shoulder-girdle and upper arms, becomes noticeable as a rule during puberty, and progresses much more slowly. It affects both sexes. And a limb-girdle type—which resembles the Duchenne but is not as rapidly progressive—is found in both children and adults.

MD confronts the scientific investigator with many puzzling questions. What is the hereditary mechanism that kicks off the disease process? Why does the period of onset vary so widely? Does some error in fat metabolism cause the fat deposits in PMD? Why does one form of the disease gallop, while another advances in slow motion? Why are different muscle groups involved to begin with, although in time all the voluntary muscles are affected?

In the *myotonias,* where a defect in the muscle membrane is known to exist, there is characteristic difficulty in relaxing the muscle after contraction. A child, for example, may find it hard to let go of an object after gripping it, and spasms may accompany the attempt. These diseases, too, reveal a familial tendency. Although *myotonia congenita,* which is usually a mild, life-long disability, is not progressive, the more common *myotonia dystrophica* is. Its relentless muscle-wasting may ultimately affect the heart.

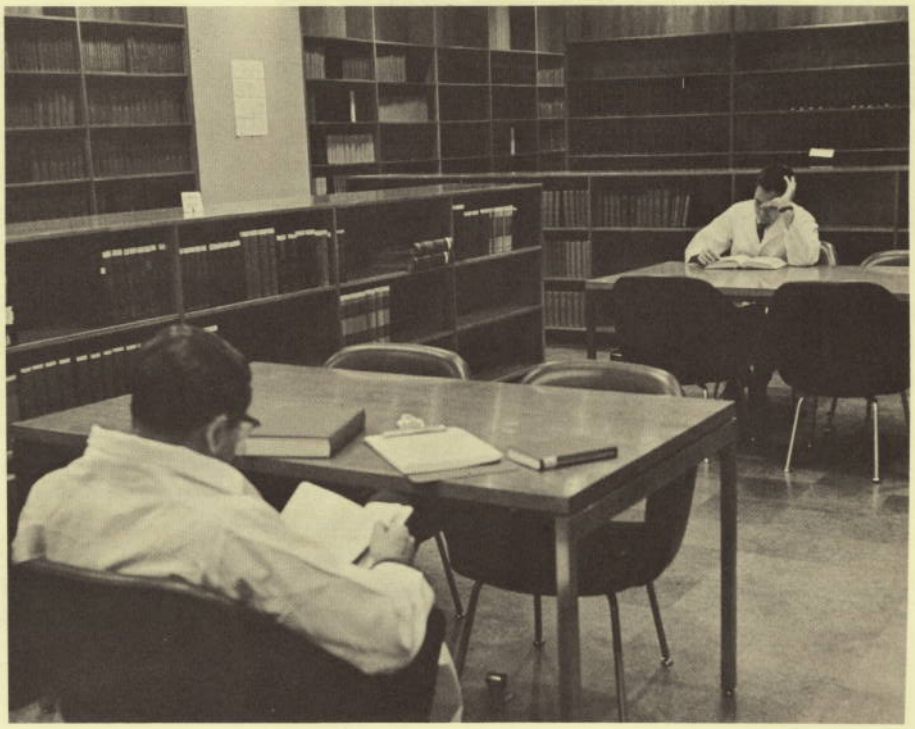
In *myasthenia gravis,* on the other hand, both muscles and nerves remain intact. The fault lies solely in the neuromuscular junction, which fails to transmit nerve impulses effectively. The result is chronic muscle weakness but often no atrophy.

The functioning and integrity of muscle are attacked also by a number of neurological diseases. In *amyotrophic lateral sclerosis,* for example, lesions in the myelin (nerve sheath) of the lateral columns of the spinal cord distort or block nerve impulses, particularly to the lower extremities. Spastic irritability and increased reflexes of the leg muscles accompany the resulting muscular atrophy. In both *amyotonia congenita of Oppenheim* and *infantile muscular atrophy of Werdnig-Hoffman* (which are probably the same disease, one beginning before and the other after birth), the anterior horn cells are degenerated and the motor nerve end plates are absent altogether from muscle. This is one of several differences in neurological as distinct from primary muscle diseases.

Many other disorders too numerous to mention here produce muscle weakness and can lead to muscle atrophy. So can certain deformities and injuries.

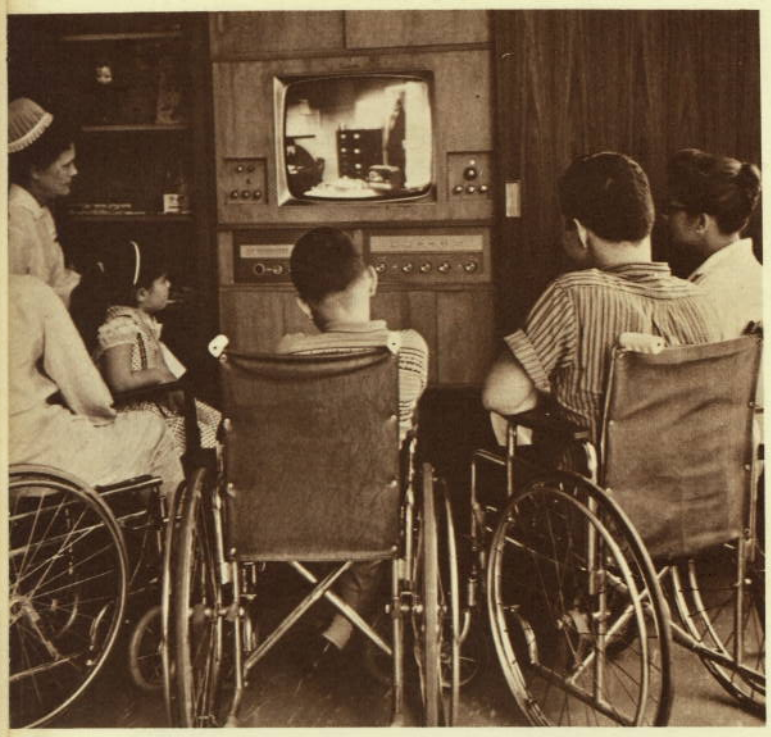
The attempt to devise effective treatments for all these afflictions might be considered a problem in applied research, but it is far more than that. Muscle is fundamental: we begin life in a bag of muscle, the womb, depend on muscle for our very heartbeat and breath. Without muscles, we could not walk, talk, chew, swallow, cough, sneeze, focus our eyes or carry out most of our daily tasks. Probing the structure, chemistry and physiology of muscle, therefore, encompasses biological research of a basic nature. So basic indeed that the scientific knowledge it keeps adding to our stockpile may well lead to relief for sufferers from many diseases besides those of muscle. At the Institute, for example, protein chemists are studying a dipeptide found in the urine of arthritics as well as of dystrophics; a medical team seeks an understanding of heart muscle metabolism which may be of use in managing cardiac cases; and genetic investigations now under way may yield clues to control of a number of hereditary diseases, and even perhaps of cancer.

Research in muscle pathologies stands now where polio research did about twenty years ago. It is gaining in scope and momentum. "We now have available techniques without which this research could not have been done before," the Institute's director, Dr. Ade T. Milhorat, has said, "and we think and see in terms not possible before . . . Our knowledge of genetics is expanding with explosive rapidity, and even the most conservative scientists must agree that herein will lie the conquest of many inheritable diseases . . . We stand on the threshold of tremendous discoveries."



Research data from all over the world finds its way to the Institute's library, which serves as a clearing house for information on muscle pathology.

Patients who serve science



The TV set in the lounge of the Metabolism Unit is a focus of attraction for both patients and their attendants. There are many other facilities for diversion in this bright and cheerfully-decorated room whose huge picture window affords a panoramic view of the New York skyline.

On the 10th floor, in the Metabolism Unit, the Institute's goal comes vividly to life. Here live some ten to twelve victims of the crippling, progressive diseases it has set out to conquer—children, adolescents, sometimes two or three adults.

These patients are all research volunteers, who are maintaind here at no expense to their families. (The metabolic ward is supported entirely by the Tall Cedars of Lebanon.) At the outset, before any long and costly investigation is begun, they are given a week's trial to see if they can take the institutional life, especially the strict diet which is essential for the metabolic tests they are to undergo. One or two have decided within a few days that they didn't after all care to go through with it. Those who remain seem to share the dedication —as an adult patient put it—of "a religious order." Under Dr. Milhorat's inspiring leadership, they regard their participation as a privilege. Two of the teen-agers have been "eating for science" from two-and-a-half to three years.

This persistence in submitting their bodies to scientific investigation demands a quiet heroism not many healthy people could match. For despite the skill and imagination of the dietary staff, who can serve up an egg in ten different versions, the diet *is* rigorous—severely controlled both in content and quantity. Every individual portion of every food is meticulously weighed, and patients must lick their plates, Emily Post notwithstanding. Any backsliding, a single candy between meals, would hold up the research for at least three days.

To offset the burden of this stern regime, the staff tries to avoid other types of control as much as possible. Visiting hours for the patients' fami-

(1) Dieticians often join patients at meals in order to keep them company and watch their reactions to some new way of serving the food in their rigidly-controlled diet.

(2) In the home-like surroundings of the recreation lounge, one of the younger patients broods over a construction problem.

(3) Patients spend several hours a day in occupational therapy. Some have developed hitherto unsuspected talents; all benefit, physically and psychically, from the activity involved.

lies and friends are practically unlimited, and there is a wide choice of recreational activities. The lounge is equipped with TV, a record player, and a large collection of table games. On the 9th floor an occupational therapy unit, maintained by the Manhattan Chapter of MDAA, offers instruction in various arts and crafts, which help to keep still intact muscles functioning. Rather than directly supervising the children, nurses keep tabs on their activities through an ultra-sensitive intercom system, which also enables patients to ask for help wherever and whenever they wish.

Fresh colors used at strategic points in the patients' quarters serve to minimize the institutional atmosphere, and panoramic views of New York's tall buildings from the wide windows add a feeling of spaciousness. Still, this *is* an institution; for respite, when a test series is concluded, a patient may go home for a week or two—usually for Thanksgiving and Christmas, too.

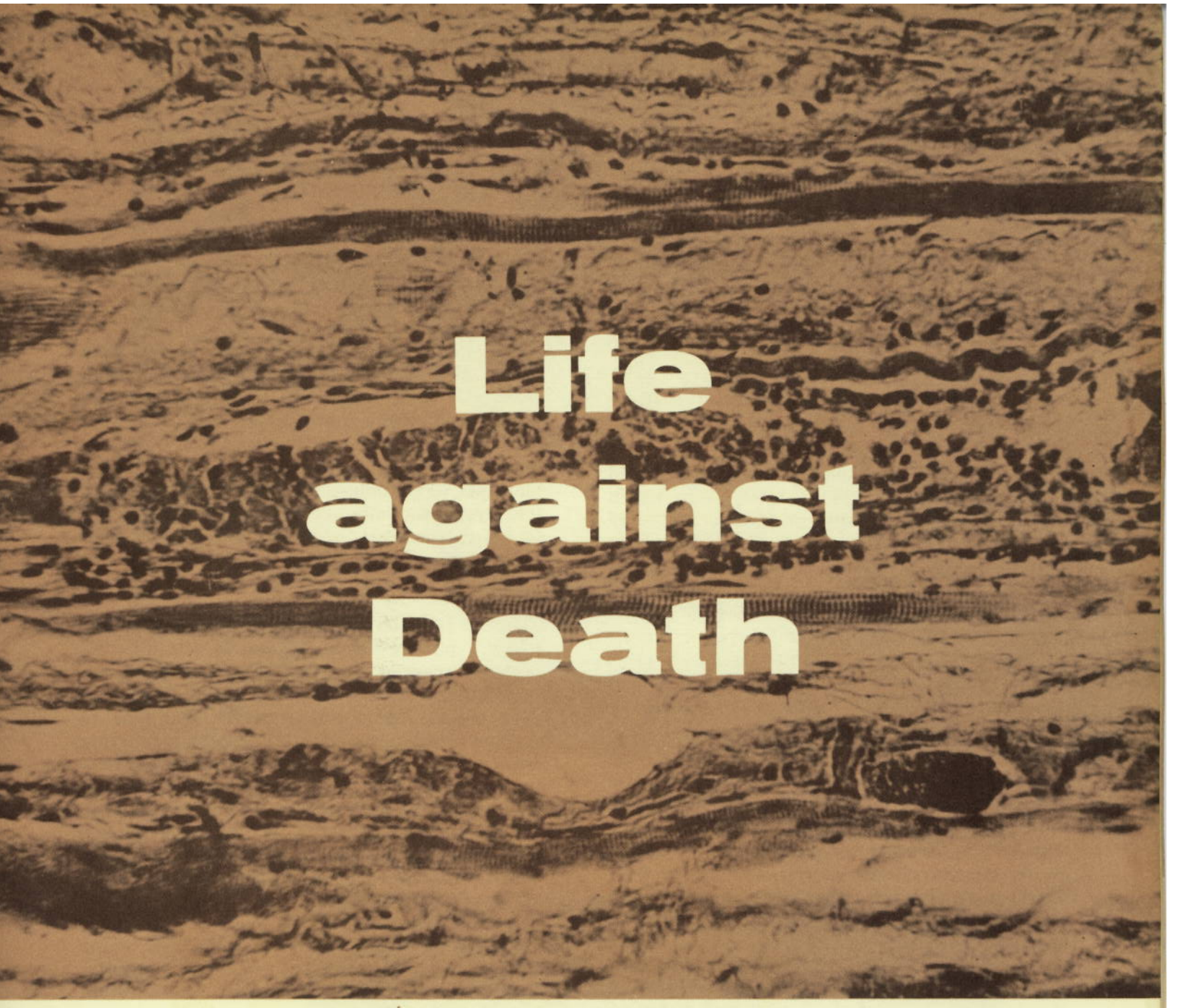
Research work with patients is centered in the Division of Clinical Investigation, which is under the personal supervision of Dr. Milhorat. The 10th floor metabolism laboratory, directed by a biochemist, makes a preliminary analysis of all specimens taken from patients. But specimens are also routed, as special studies may require, to laboratories throughout the building.

All the food the patients consume is chemically analyzed. To avoid costly and time-consuming repetitions of this work, samples of a big batch of, say, string beans from Long Island or potatoes from Maine are analyzed at one time, and the batches are then stored in the deep freeze room.

Only when a patient's diet is constant, containing known amounts of carbohydrate, protein, fat, vitamins and calories, can the effects of adding one specific compound to it be assayed. This is done through analysis of blood and excretions. In various muscle-wasting diseases, enzymes needed for metabolism leak out of muscle cells, and consequently unmetabolized substances accumulate in the blood and urine. The plainest indication of such disturbed muscle metabolism is reduced urinary output of the normal waste product, creatinine, and high urinary output of unused creatine. A change in the balance of creatine-creatinine in urine therefore provides direct evidence of the effect of a preparation, whether a vitamin, hormone or other compound. Indirect evidence can be gathered in other ways, but the creatine-creatinine ratio remains the most satisfactory gauge.

It would be gratifying to be able to add that this test had proved one compound or another to be beneficial. Although many substances found to stimulate muscle action in animals without harmful effects have been administered to patients, the sad truth is that so far none has had any lasting therapeutic effects.

A young patient watches with interest as Dr. Andre C. Kibrick, in charge of the metabolism laboratory, prepares for an experiment.



Life against Death

To chart the course that leads to the end-of-the-line clinical tests on patients, several kinds of scientists must bring their particular approaches to bear on the problems of muscle. They represent not medical specialties alone, but physiology, cell biology, protein, physical and biochemistry, enzymology and genetics as well.

Physiology

Concerned as he is with living processes, the physiologist wants to know how intact muscles work. The Physiology Division therefore concentrates on the study of muscular activity under conditions as close to life as possible.

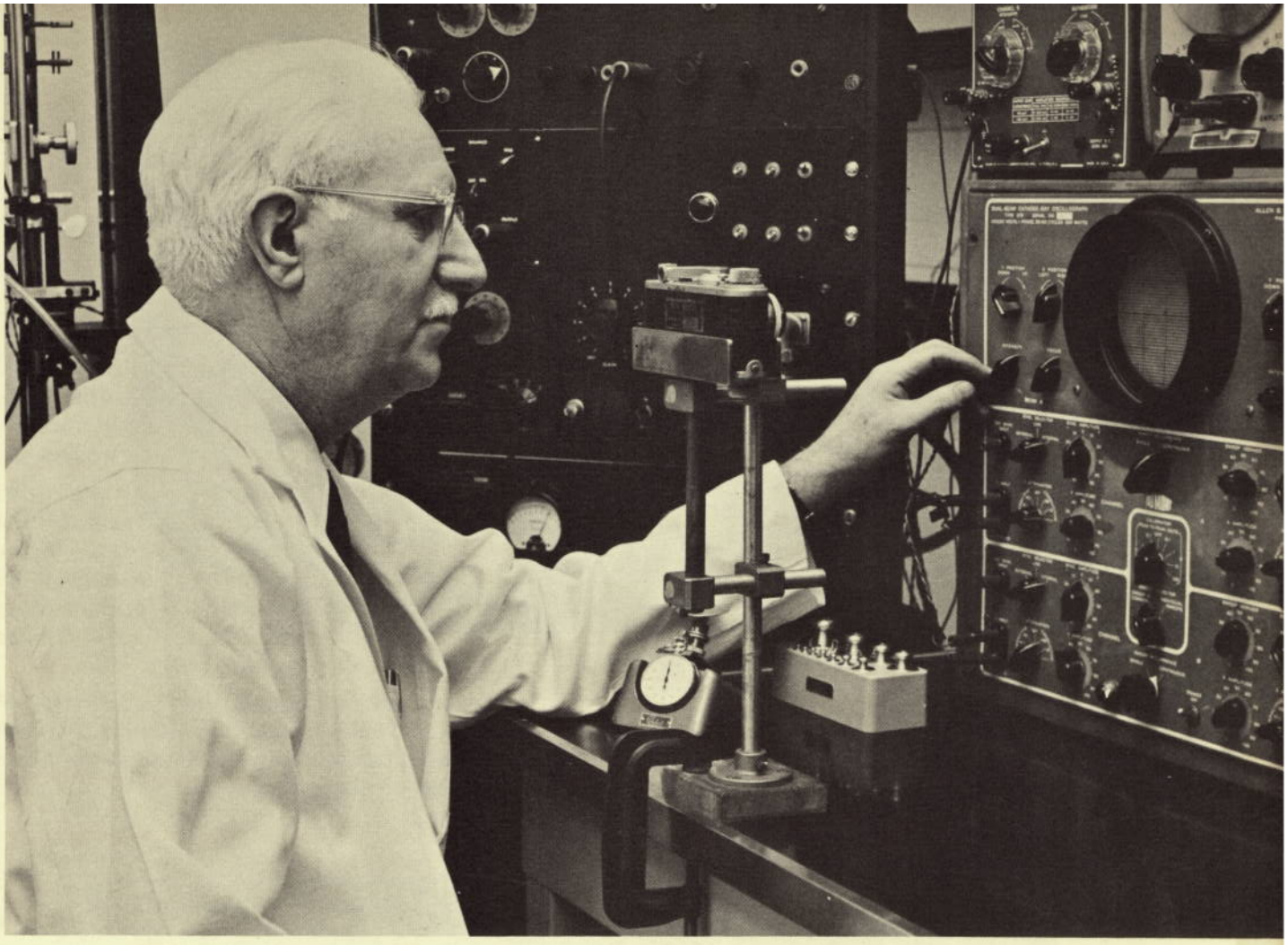
After excision from the body, properly treated muscles, and even individual muscle fibers, retain their vital functions surprisingly well for many hours. IMD physiologists use such excised muscles to study the role of the action potential (the electrical change that occurs in the membrane of a stimulated muscle fiber) in activating the contractile filaments within the membrane. Of special interest are the effects of caffeine, and of quinine, nitrate, zinc and uranyl ions. Although they influence the action potential in rather different ways, they all increase the strength of contraction.

Zinc is notable in this research because it is an indispensable element—animals deprived of it lose hair and develop a splotchy skin, and young ones show stunted growth. Although minute concentrations of zinc increase the contractile response of excised frog muscles to electrical stimulation, IMD physiologists have found that larger concentrations completely block the nerve impulse at the neuromuscular junction. Could faulty metabolism of zinc, permitting it to accumulate to harmful levels, cause the junction defect in myasthenia gravis? Muscle contains much of the body's zinc, and some inadequacy in its use may also play a role in muscular dystrophy. Both these possibilities are being investigated by the Physiology Division.

An unexpected finding in experiments with mouse muscles is that the dystrophic ones, though relatively weak, fatigue much more slowly than the normal. Present studies seek to determine whether this is due to differences in the action potential, the chemical systems of the contractile filaments, or the influence of the former on the latter. Dystrophic mice are also under investigation to see whether parts of the reflex structure, especially certain muscle sense organs, are affected by the disease.

In the Division's research in "molecular physiology," pure extracts of muscle — contractile proteins, energy-containing substances, salts (especially of calcium), membranes and other components—are put together in the test tube in various simple reconstructions that mimic living muscle. Study of such extracted systems is revealing much about how live muscle uses calcium in bringing about relaxation as well as contraction. An understanding of the relaxation mechanism might make it possible to relieve the permanent contractures of advanced MD patients.

In our voluntary muscles much of the body's protein is concentrated. Aside from their water content, they are 80 per cent protein. The physical and chemical properties of protein are therefore key areas to be charted.



Dr. Alexander Sandow, chief of the Physiology Division, at the dual beam cathode-ray oscillograph, an apparatus which can measure muscle responses lasting only a fraction of a thousandth of a second.

A molecular physiologist, Dr. Annemarie Weber, adjusts the K distillation apparatus which is used for nitrogen determination.

Physical Chemistry

Investigation of the physical properties of muscle proteins in solution goes on apace in the Institute's Physical Chemistry Division. One of its major projects is to observe and photograph the migration of protein molecules in an analytical ultracentrifuge, a $30,000 precision instrument which can revolve at speeds up to 60,000 RPM. Since heavy molecules migrate faster than lighter ones, these experiments provide a basis for calculating molecular weights, which is one of the main purposes of the machine.

In another key type of study, light is passed through a solution of muscle proteins. The bigger the dissolved molecules, the more light they scatter. Measurements, with photo tubes, of the light scattered at several angles enable the investigator to figure molecular size and shape, as well as weight.

IMD physical chemists hope in time to supplement these studies with investigations of the internal structure of the protein molecules—to find out whether they are arranged in a helical (like a coiled spring) or random pattern. Such a project would require the acquisition of a $13,000 instrument called a spectropolarimeter.

Somewhere, sometime, these exhaustive observations and calculations may turn up a significant *physical* difference between the proteins of normal and those of diseased muscle.

The molecular weight of muscle proteins can be determined by means of the light-scattering photometer shown above. Dr. Hans Oppenheimer, chief of the Physical Chemistry Division, notes down the results.

The Protein Chemistry Division is headed by Dr. Charlotte Ressler, shown adjusting a distillation column in her laboratory.

Protein Chemistry

The Institute's protein chemists are studying amino acids and peptides (the building blocks of protein) which occur naturally in plants and animals. They have isolated several, determined their chemical properties, synthesized them, and investigated their biological activity in animals. In particular, they seek to understand the role of peptides in muscle function.

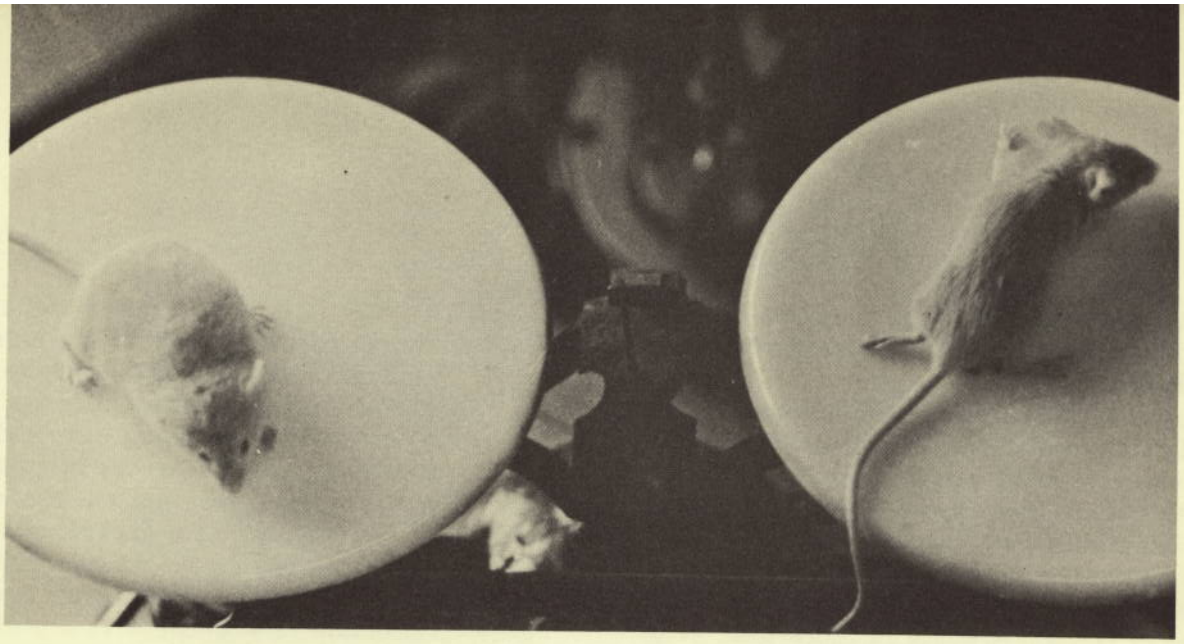
One ongoing investigation may have already yielded a clue to the origin of a crippling and sometimes fatal neurological disease prevalent in parts of India and the Mediterranean. Since it has been associated with the consumption of peas from various lathyrus plants, the disease is called *lathyrism* or *neurolathyrism* and has been thought to be due to a dietary deficiency. But it may stem from the presence of a toxic agent. The chief species of pea plant suspected is harmless to laboratory animals, but the seeds of common vetch found as a weed among samples of the peas collected from various parts of India have proved injurious to the nervous system of ducks and monkeys. Institute chemists have succeeded in isolating a toxic factor in the vetch seeds, and have found it to be identical with an amino acid which they had already synthesized —**β-Cyano-L-alanine**. They have shown both natural and synthetic versions of this substance to be harmful to chicks and rats; doses of 11 milligrams and 20 milligrams per 100 grams, respectively, are fatal. Whether the biological effects are similar in man remains to be determined. Nor can one ignore the possibility that other nerve poisons may be present in the vetch seeds and implicated in *neurolathyrism*.

Nevertheless the **β-Cyano-L-alanine** findings are provocative. Is this amino acid present in and absorbed from other foods? It is closely related chemically to asparagine, which is present in human blood and tissue. Does the body sometimes make a slight error in synthesizing or utilizing asparagine, and so turn it into a nerve poison?

Contractile Proteins

When muscles go into action, they contract; in speaking, for example, our throat muscles contract up to 1,500 times a minute. Contraction requires, in addition to structural proteins, an intricate sequence of metabolic steps for converting food into mechanical energy. The final step, in the muscle cells themselves, is the interaction of the related proteins, actin and myosin, with adenosine triphosphate. Using isotopic methods, including the radioactive "labelling" of substances, one IMD investigator is seeking more precise knowledge of this interaction and the conditions which hamper or facilitate it. He wants in particular to pinpoint the sites and chemical character of the so-called "active centers" of the protein molecule—those infinitesimal portions of its surface which participate in this or that chemical reaction. Materials found to inhibit the interaction of actin, myosin and adenosine triphosphate in the test tube are tested on living muscle fibers to see if they also block muscle contraction. In this line of research lies the hope of identifying some abnormality in the cells of diseased muscles which interferes with contraction—an abnormality either in the contractile proteins themselves or in the cell components immediately responsible for their activity.

Dr. Michael Bárány, head of the Contractile Protein Division, and his wife, Dr. Kate Bárány, who specializes in physical chemistry. The instrument is an analytical ultracentrifuge.



Mice from an inbred strain, in which dystrophy arose as a spontaneous mutation, provide an invaluable tool for researchers. Special breeding techniques are necessary to make them available in sufficient quantity to meet the demand.

Enzymology

The abnormality may not be in the muscle cells, however. It may be at some earlier stage of the metabolic process which begins in the stomach and lungs with the intake of food and oxygen and goes through many of its most intricate steps in the liver, the body's principal chemical laboratory and storehouse. Each metabolic step (whether the transport of oxygen or ions or the transfer of an atom or molecule from one compound to another) is controlled by a chemical governor called an enzyme—a specialized protein molecule whose sole function is to regulate one particular chemical action. The activities of these biological catalysts are influenced by certain of the vitamins, hormones and minerals, deficiencies of which can seriously impair enzyme functions.

For some years, it has been known, for example, that animals fed a diet deficient in vitamin E suffer rapid muscle degeneration similar to muscular dystrophy in man. When the vitamin is restored, they recover just as rapidly. Massive doses of vitamin E unfortunately have no effect on human dystrophics, who already have ample supplies of it which they are apparently unable to utilize. Nevertheless, continuing studies of vitamin E-deficient animals are providing valuable information on the metabolism and biologic role of this vitamin and of substances structurally related to it.

The membrane of normal muscle cells is highly selective, admitting needed materials and barring unwanted or potentially harmful substances, allowing the exit of waste products but retaining all important molecules, such as enzymes. In muscle-wasting conditions, however, the membrane becomes abnormally permeable, and enzymes that should remain in muscle leak out.

They are then found in the blood in unusually large amounts. Two of these "lost" enzymes are aldolase, concerned in the metabolism of carbohydrate, and creatine phosphokinase, concerned in the metabolism of creatine.

Abnormal amounts of aldolase have been found not only in the blood of dystrophic children before their muscle difficulties have become pronounced, but also in the blood of some apparently normal mothers who have transmitted the disease to their sons. If such a metabolic alteration in women who are themselves free of dystrophy but are carriers of hereditary MD should be widely confirmed (as it has not been as yet). it would provide a means of detecting the carrier state.

In normal persons, creatine is metabolized by the voluntary muscles (its chief repository), and the resulting waste product, creatinine, is excreted in the urine. In fact, the amount of creatinine excreted daily on a diet free of this substance is a fair index of an adult's total functional mass of muscle. But where muscles waste, the creatinine excretion diminishes, while more and more unused creatine appears in the urine. This inbalance also accompanies muscle degeneration produced by a deficiency of vitamin E, but is quickly erased when the vitamin is restored. On the other hand, disturbances of muscle function where there is no wasting as in myotonia congenita, myasthenia gravis or temporary paralysis do not reverse the creatine-creatinine ratio in urinary output. Hence the measurement of creatinuria is a valuable diagnostic tool. And, as we have seen, it also serves as a yardstick for determining the therapeutic usefulness of chemical compounds tested on patients.

One Institute biochemist has investigated a number of enzyme systems in the muscles of vitamin E-deficient rabbits and of mice with a hereditary muscle disease similar to human dystrophy. Along with diminished levels of several enzymes needed for normal metabolism, he found increased levels of cathepsins and other hydrolytic enzymes that degrade protein. These findings are in line with earlier studies of amino acids tagged with radioactivity which showed that the muscles of the dystrophic mouse build new protein at *twice* the normal rate, but break it down *three times* as fast, an imbalance leading to the loss of muscle tissue which is the core problem in MD. Are abnormally large amounts of these proteolytic enzymes produced, are chemical substances that normally inhibit their activity lost, or are other substances that increase it made available? These are further questions for the researcher. A few clues already turned up support the hypothesis that enzymes normally localized in a relatively inactive form in muscle are somehow "triggered" into becoming very active protein-degrading agents.

Rabbits are among the many animals used in muscle research. Here a technician weighs one specimen while Dr. Irwin Weinstock, of the Enzyme Chemistry Division, checks the results.

Dr. George Acs, head of the Division of Enzymology, at the console of the scintillation counter. The instrument is used to measure the radioactivity of tissue samples.

Genetics

Since each of the many thousands of enzymes in the body is probably linked to a specific gene, research problems in enzyme chemistry inevitably spill over into the field of genetics. Genes, the heredity-bearing units of the chromosomes present in every living cell, may spontaneously change in structure and therefore also in influence. Such mutations may be beneficent, leading to new and more effective adaptations. Or they may produce a disease or disability which can be transmitted to later generations.

A mutation of the latter kind occurred in the Jackson Memorial Laboratory at Bar Harbor, Maine, where mice of a known genetic strain began to appear with a disease similar to human MD. Since 1955, when their condition was identified, the crippling gene has been transmitted through many generations, affording researchers opportunities of probing the secrets of muscle disease in ways not possible with human patients. Inherited dystrophy has likewise appeared in a strain of New Hampshire fowl, which are especially valuable for research because the two types of muscle fiber, the red and the white, are more clearly separated in chickens than in most other species. Red and white fibers are known to differ in their activity and chemical composition, and they respond to the dystrophic process at different rates. Research using dystrophic chickens may therefore help to reveal why the onset of different forms of the disease involves different muscle groups in man. In the exploration of hereditary disease mechanisms, both dystrophic mice and dystrophic chickens are being extensively studied at the Institute.

Just how a mutation may give rise to muscle disease is not yet known. The changed gene might fail to direct the synthesis of its corresponding enzyme, produce it in inadequate amounts, or bring about an imperfect synthesis. In any of these situations, the step-by-step chemical reactions which we call metabolism would be interrupted at some point. The resulting chemical derangement could impair muscle tissue in more ways than one.

A known impairment, for example, is the lack of a readily usable sugar, glucose, in muscle, due to the genetic absence of an enzyme, one of the phosphorylases, needed to change glycogen (the storage form of sugar) into glucose. Another kind of damage occurs when some chemical substance which is normally present in the body in small amounts is not used in the metabolic process and consequently builds up to harmful levels. This is

what happens in *phenylketonuria*, a metabolic defect which brings on serious disturbances of the nervous system. If scientists could learn the precise nature of an enzyme failure in muscle metabolism, they might be able to devise a compensation for it, somewhat as doctors now routinely compensate diabetics with insulin.

An even more stirring possibility is that of intervention to correct or compensate for the faulty genetic "code." A gene, which can be defined only in terms of its effects, is believed by some geneticists to be a segment of a molecule of DNA (deoxyribonucleic acid). DNA, the chemical material which is found only in the cell nucleus, has been identified as the primary bearer of hereditary instructions. Its close chemical relative, RNA (ribonucleic acid), which is found both in and out of the nucleus, seems to act as a kind of template, stamping the DNA directives into the enzyme proteins as they are manufactured in the cell.

Exactly how all this is done and which parts of the RNA are responsible are still matters of scientific investigation and controversy. But the composition of the RNAs of various species of living organisms is becoming known in greater detail, and differences between the RNAs of normal mouse muscles and those of mice with hereditary form of dystrophy have been demonstrated at the Institute. Similar investigations in normal and dystrophic human beings must be carried out before any therapeutic applications of the new nucleic acid knowledge can even be considered. But it seems certain that attempts will eventually be made to alter abnormal RNA with the aim of enabling it to produce its regular complement of enzymes. Here is a frontier of biological research which opens up breathtaking vistas of a future in which man may learn to influence his own evolution.

Wasted muscles prevent this chicken from righting itself when placed in a supine position. It belongs to a strain of New Hampshire fowl in which dystrophy appears as an inherited characteristic.

Biochemistry of Trace Elements

Hertha T. Taussky, shown repairing a combustion chamber igniter, heads the division concerned with the biochemistry of trace elements.

In recent years it has been discovered that minute traces of certain elements in the diet are essential to the healthy functioning of both animals and man. Investigators found that laboratory animals developed liver necrosis after being fed Torula yeast, while those fed brewers' yeast flourished. The main difference between the two yeasts turned out to be the presence of selenium in the brewers' type. In New Zealand, which has very little selenium in its soil, farm animals were born with a muscular weakness which was cured by adding selenium to their diets. Moreover, when combined with selenium, less vitamin E is required to restore animals with vitamin E deficiency to health.

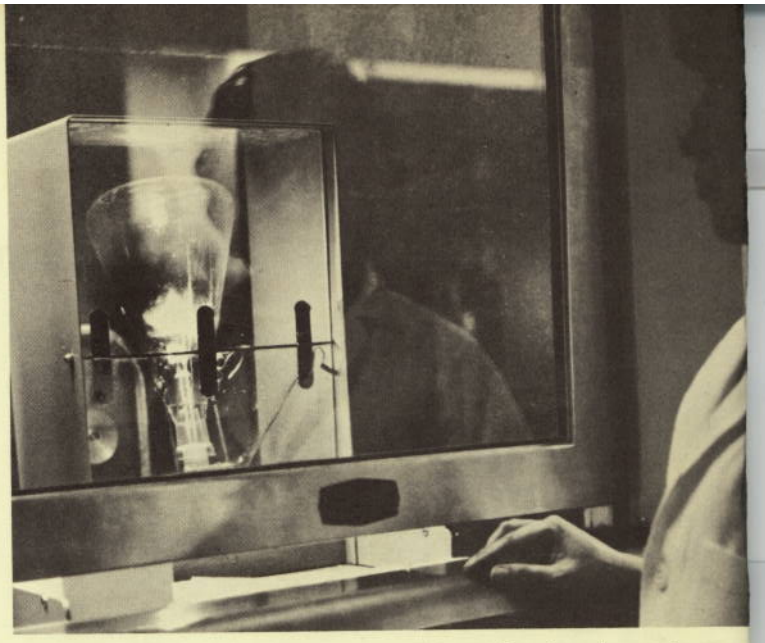
These discoveries might suggest that selenium is a simple remedy for muscle disease. But it is not. Amounts as minute as one part in 20 million parts of the diet can prevent or cure muscle degeneration in several species of farm animals. Only slightly larger but still minute amounts, such as one part in five million, are poisonous. Before risking this double-dealing substance on patients, much more must be learned about its metabolism in animals and man. The Institute program therefore includes studies of the biological role of selenium in maintaining the integrity of voluntary muscle.

Periodically the diet kitchen staff cooks a duplicate of a patient's diet for a full day. After homogenizing this food sample, a biochemist subjects it to chemical analysis to determine the patient's natural day-to-day selenium intake, which comes mainly from cereals and eggs. Selenium intake can then be compared with selenium excretory output. Together with analysis of various tissue extracts, this intake-output ratio may shed light on the biochemistry of selenium.

Investigation of trace elements in living organisms poses unique technical problems because the quantities involved are so minute that they are easily lost in the process of chemical analysis. The scientists overcame this difficulty by devising a new

application of the Schöniger combustion method. In a closed, oxygen-filled flask, the material to be analyzed—such as muscle fiber, kidney tissue (in which selenium tends to accumulate), egg shell, yolk or white—is burned, with an appropriate liquid in the bottom of the flask absorbing the ash. The material can then be studied in solution without loss of the volatile selenium.

Along with these chemical analyses go experiments with dystrophic chickens and their eggs. Varying dosages of selenium have been tried out in their diets and injected into the eggs. So far the results, although interesting, are not conclusive.



A sample of human tissue, containing a fraction of a part per million of selenium, is reduced to ash in an oxygen-filled combustion flask.

Myocardial Metabolism

Although the heart muscle is not one of the voluntary muscles, it is, like them, a striated (banded) muscle. (Other involuntary muscles appear smooth under the microscope.) When the heart muscle becomes affected, as it sometimes does, in advanced stages of muscle-wasting disease, this can be a matter of life or death for the patient. Some way of detecting heart involvement in its earliest phase could be of vital importance. Investigators in the Institute's myocardial metabolism division are measuring and comparing the time intervals of heart action and rest in normal persons and dystrophic patients. If they find that, in certain patients, the action time is unduly long and the rest time too short, a recognition of this condition could serve as a timely warning to relieve strain from the heart.

The division also seeks to find out whether sugar or fat is more important in metabolism of the heart muscle. The answer to this question, as well as information from the heart action time studies, may be of help in the management of arterial and other degenerative diseases of the heart, as well as in muscle disease.

New Tools for Research

Many biological discoveries would not have been possible without the new tools which have been put in the hands of research workers in recent years.

The Institute's electron microscope, a $40,000 instrument in the Cell Biology Division, is an invaluable aid to all members of the scientific staff who are engaged in studies at the cellular level. With its capacity to magnify a given area up to 100,000 times, and a built-in camera offering a means of photographic recording and further enlargement, this instrument enables investigators to "see" the cell about a million times larger than life. Even more important than its magnifying power is its capacity to resolve (distinguish as separate from one another) very minute objects which are very close together. Where the conventional light microscope can resolve objects about 2,000 angstroms apart (an angstrom equals one hundred-millionth of a centimeter), the electron microscope, when pushed to its limits, can separate out objects only 6 to 8 angstroms apart. (Revolutionary modifications in the design of such instruments are now undergoing trials at the University of Arizona and other research centers in an effort to increase this resolving power to 2 angstroms—the distance between one atom and the next.)

Using new techniques of electron microscopy, some of which they have themselves developed, Institute cell biologists have examined hundreds of specimens of muscle removed at operations in the nearby hospitals. They have studied the muscle membrane, the motor nerve end plate, the nucleic acids, the proteins, the glycogen, the lipids and the enzymes in the muscle cells. They have thus been able to test by direct observation many conclusions which previously rested only on inference.

Even with the ordinary light microscope it has been possible to detect some structural differences between normal muscle and muscle in advanced disease states. But since voluntary muscles react to harmful influences in only a limited number of ways, the microscopic picture in the later stages of wasting looks much the same, whatever the initial cause. With electron microscopy it may be possible to identify pathologic changes much earlier. Where the light microscope shows a cross section of muscle fibril looking like an end-on view of bunched finger tips, the electron microscope presents a lacy network of fibers, punctuated at their intersections by small round bodies called mitochondria. These complex structures contain enzyme systems. In muscle biopsy specimens taken from young PMD patients, the mitochondria appear swollen. Several different biologic and metabolic disturbances have been shown to cause swelling of the mitochondria and alterations in enzyme function are known to accompany this manifestation. Study of such early pathological changes may in time uncover different primary sites of the disease process in different types of muscle disorders.

A powerful tool of the protein chemist is the $14,000 amino acid analyzer—a miniature chemical laboratory with some of the features of an electronic computer. In twenty-four hours this tireless robot chemist can provide a full amino acid analysis of a sample of peptide or protein hydrolysate, a physiological fluid or tissue extract—results which formerly took weeks of laborious work to obtain. When radioactive isotope elements are to be traced, the analyzer is hooked up with a Geiger counter. Completely automatic, it delivers a continuous record of its operations on a paper strip, and shuts itself off when the job is done. Thus it can be left to work by itself overnight or over a week end.

Another automated servant of science is the $14,000 scintillation counter, which is used to measure the radioactivity of liquids or tissue samples in test tubes. One type of counter handles solids, another liquids. A revolving turntable feeds the test tubes containing radioactive samples one by one into the machine which counts and records the number of radioactive pulses or scintillations each gives off in a specified time. The machine can be set to measure each sample for 30 or 60 seconds or for whatever time interval is desired. Meanwhile, the console flashes a record of the passing seconds and tenths of a second and the number of scintillations, much as a gasoline pump records gallons and fractions of a gallon and prices. This record provides the most sensitive measure of enzyme reactions and the resulting synthesis of chemical compounds.

When IMD scientists cannot find commercially-produced machines to serve their purposes, they can turn to the Instrumentation Center for help. Here an electronics technician and a machinist, under the direction of an electronics engineer, design and build equipment to the scientists' specifications.

The measurement of muscle contractions, for example, poses special difficulties. Transducers on the market (instruments for converting mechanical motion into electrical signals which can be "read" on a cathode-ray oscilloscope) are in general quite sensitive enough, even to picking up the tensing of only a single muscle fiber about an inch long and no thicker than a fine human hair. The problem the Instrumentation Center had to solve, however, was the utilization of such high sensitivity to record the muscle's contraction with perfect fidelity and without blemish by extraneous mechanical disturbances. To eliminate disturbing draughts, for example, the hypersensitive assembly was enclosed in a plastic cage, from which wires feed out to the electrical units controlling the experiment and to the oscilloscope, whose screen records both the mechanical and the electrical responses of the muscle fiber. Lest outside vibrations should distort these results, the entire apparatus was anchored to a concrete slab supported by springs and insulated by rubber pads. A camera focussed on the screen makes a film record for future reference.

Other equipment built at the Institute includes an automatic dialyzing unit (for the separation of crystalloid and colloid substances), emulsion rollers of very fine tolerance for measuring calcium in muscle, and a small microradiography unit. Maintenance and repair work on the vast array of mechanical, electrical and electronic equipment in IMD laboratories is another function of the Instrumentation Center. Long-range plans include the building of an automated data analysis and control unit for physiology experiments. After computing the results of one experiment, this unit would immediately set up conditions for the next and start it rolling. Such a timesaver would make it possible for the physiologist to conduct a series of experiments on a single specimen of muscle in the short time it can be kept alive.

Dr. Ahmad Shafiq is a member of the Cell Biology Division. He is shown here at the electron microscope which is capable of magnifying objects up to 100,000 times their actual diameter.

"Actions Are Our Epochs"

The scientific detective story has not yet reached its climax; the muscle-cripplers and killers have not yet been traced. The search must go on—at the source of hereditary attributes in the nucleic acids, over the whole length of the metabolic pathways, and in the intimate mechanisms of muscle itself. Even the dead ends must be explored and crossed off as such.

Often one is impatient for results. But they cannot be forced. A physician eager for a new healing aid once put pressure on a biochemist now at the Institute to validate a reputedly promising compound for treatment purposes. "We get some good results, but some are not so good," the chemist protested, "and we don't yet know why." "You've got to cross the road some time," the doctor urged. "But not when there's a red light," was the firm reply. Mindful of Hippocrates' warning, "It is more important to do no harm, than even to help," no responsible biological scientist wants to exploit a finding until he has made absolutely sure of its validity.

And yet for a layman, even if he cannot grasp all the meanings, to sit in on a seminar as IMD investigators report on their work is to catch a glimpse of medical history in the making. The Institute has been aptly called a "tower of hope," but it is more than that. It is a tower of action—strong action in a major offensive against the dystrophies, the myotonias, myasthenia gravis and all the other muscular and neuromuscular afflictions. If actions are our epochs, as Byron said, then this action may usher in an epoch marked by the conquest of these dread diseases.

INSTITUTE FOR MUSCLE DISEASE

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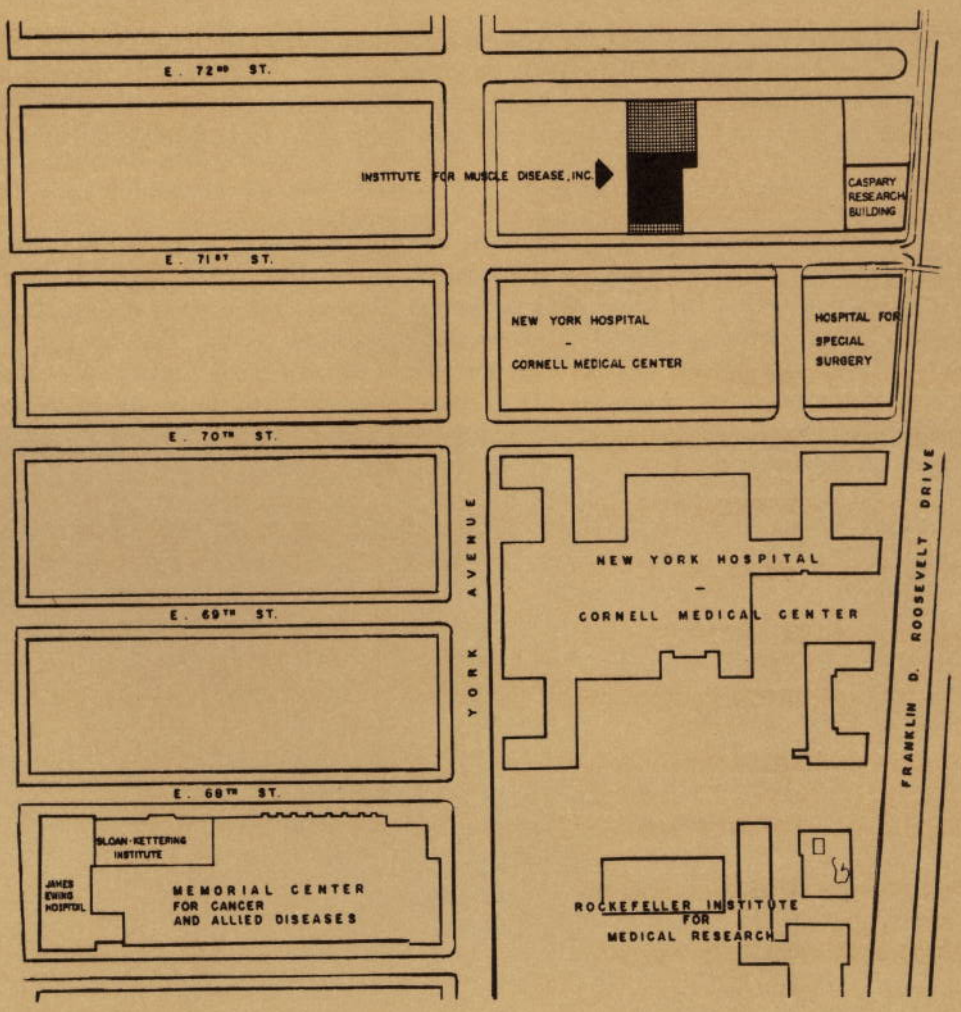
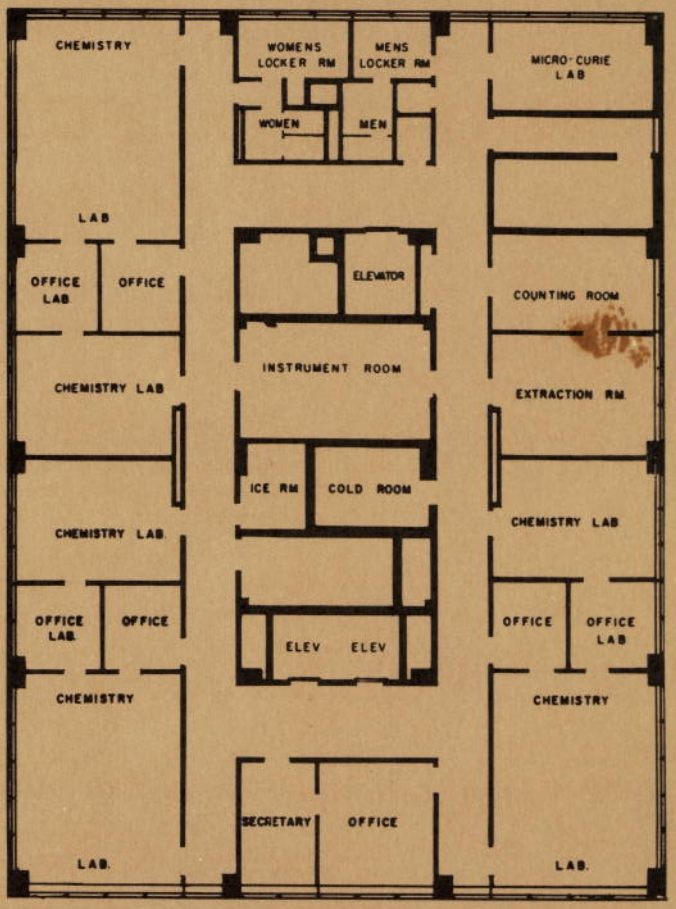
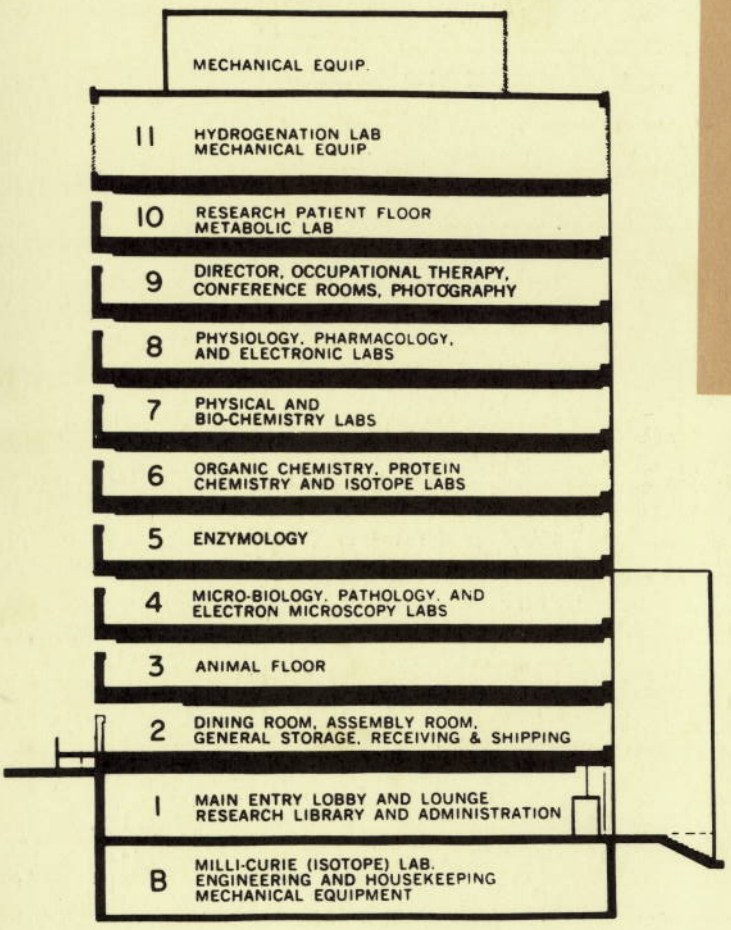


The Institute in relation to the great medical community of which it forms a part.



A typical laboratory floor.



Profile layout of I.M.D. facilities.

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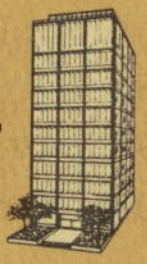

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