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The Physical Basis of Life and Learning

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Thomas Henry Huxley, on 18 November 1865, read a paper, in Edinburgh, entitled "The physical basis of life" (*1*) in which he strongly advocated what, at that time, seemed daring to many biologists and heretical to the lay public, to whom the defender of the evolution hypothesis appeared to be attempting to reduce life and spirit to physical mechanism. No mechanist, Huxley eschewed philosophical implications; he was advocating a frontal attack by physical methods on the properties of "living protoplasm," a mysterious watery mixture that constituted the units called "cells," identified fewer than 30 years previously, as the biological basis of life. In the same decade in which Darwin announced his generalization, the Scottish chemist Thomas Graham applied the term *colloid* to aqueous systems containing large molecules or aggregates of molecules, to distinguish them from systems of truly dissolved crystalloids (*2*). And for a later generation, in the 1920's, Sir F. Gowland Hopkins sounded the intellectual keynote of the times by referring to life as a "dynamic equilibrium of many substances in a polyphasic system" (*3*).

After sketching the present status of the enterprise advocated by Huxley, I shall here propose a similar concentrated investigation of the physicochemical processes underlying memory, learning, consciousness, and other mental processes in man. If it is not inappropriate in this historical context, I should perhaps emulate Huxley by disclaiming mechanistic philosophical connotations in what to me is no more than science. Indeed, Huxley implied a view suggested by the title of this article when in an 1868 address he said: "The thoughts to which I am now giving utterance, and your thoughts regarding them, are the expression of molecular changes in that matter of life [that is, protoplasm] which is the source of our other vital phenomena." I shall also suggest that the subject of our study is so complex that to nucleate new insights demands new kinds of organization not only of science and communications but also of scientists themselves.

Hierarchical Conceptual Impasse: Molecule-Chromosome-Organism

In the first and second decades of this century, biologists were busily engaged in consolidating the gains achieved during the epoch-making advances of the last half of the 19th century and in exploring exciting new possibilities. Cytologists were exploiting microtechnical methods in a search for the physical basis of life *within* cells at a level of size resolvable with the light microscope. Theories were developed which ascribed life-giving qualities to granules or membranes or fibers. Each of these theories attracted authoritative sponsors. In terms of present-day molecular biology, the granules are mitochondria; they are the power plants of the cell and are essential to life, but are not in themselves "living." The membranes, as endoplasmic reticulum with associated polyribosomes, are the biosynthetic center of the cell and are therefore vital, but not, as such, "alive." Chromosomes, as fibrous polymers, come closest to fulfilling the definition of "life" in that they are capable of precise replication. Cytogeneticists were providing conclusive evidence that chromosome-bound "genes" were the effectors required by Mendel's correlations, which had recently been popularized after 40 years of neglect by biologists: chromosomal sets undergo precise division in mitosis, and maternal and paternal sets segregate precisely during meiosis. Evidence favored the view that the genetic determiners must be arrayed at definite and constant intervals and in definite serial order along the chromosomes.

In 1923 the renowned cytologist Edmund B. Wilson published the first Sedgwick Memorial Lecture (*4*), honoring the eminent biologist William Thompson Sedgwick, my predecessor as professor at Massachusetts Institute of Technology. Like Huxley, Wilson chose as his title "The physical basis of life." His expository masterpiece portrayed progress made in the half century since Huxley.

The subject was vigorously debated during the summer of 1923 in the country's leading forum of biology, the Marine Biological Laboratory at Woods Hole, Massachusetts. As a student in the physiology course there that summer, I was privileged to hear the debates between the giants of the time, controversies from which we

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may well learn a lesson of value in contemporary colloquia on the physical basis of memory, learning, and the mind. Preeminent pioneers in biological and physical chemistry, such as J. Loeb and C. M. Child, ridiculed the contentions of biologists, such as T. H. Morgan, E. B. Wilson, E. G. Conklin, and C. E. McClung, that the genetic determiners are carried on chromosomes; it was plain to Loeb that only *molecules* possessed the potentialities required to select and direct the infinite number of reactions involved in embryogenesis and development. The biologists, for their part, decried the chemists' lack of knowledge of biological principles, particularly of cytogenetics, which conclusively demonstrated chromosomes to be the hereditary determiners. Both groups—the molecular reductionists on the one hand and the cellular, organismic, or naturalistic holists on the other hand—were right, and both were wrong. The molecular reductionists piled up on the Scylla of molecular componentry because their conceptual models lacked a systems-type organization in which the stored information might be meaningfully processed; the cellular and organismic holists, on the other hand, foundered in the Charybdis of reliance on mystical emergent properties of systems devoid of demonstrable information-containing molecular components. In this hierarchical molecule-chromosome-organism impasse, created by artificial professional and parochial barriers of communication, the two groups were prevented from joining forces in a common assault on the problem at all levels because the molecular effectors of genetics, now called DNA and RNA, were still uncharacterized physically and because these molecular effectors are not only molecules but also enormous linear polymers. To have suggested that DNA may have a molecular weight of 10^6 to 10^9 would have been considered sheer nonsense in 1923.

The concept of first magnitude in *molecular* science—in contrast with the great concepts of *systems*, or holistic science, which up to that time had shaped men's thinking regarding life—had not yet been formulated. It may be characterized thus: information vital to life can be stored, transferred, and retrieved in systems containing large polymeric molecules; through the virtually limitless repertoire of structural variants available in the tertiary conformation of protein molecules, by specific recognition and catalytic properties these molecules can utilize the information stored in the DNA and RNA and carry out the phylogenetic and ontogenetic instructions implicit in the DNA code.

This historical reference emphasizes the analogy to the present period, so rich in opportunities to strengthen the behavioral sciences and the neurosciences through the application of rapidly unfolding insights into the properties of macromolecules and of systems of macromolecular assemblies. The contemporary scene is also marked by professionalistic, discipline-bound tendencies to resist the much-needed interdisciplinary, ecumenical reform, even in the face of the obvious opportunities and of the urgent necessities that now confront mankind.

Molecular Determinants in Genetics and Immunology

Following the realization that DNA is a giant macromolecular polymer composed of nucleotide monomers of four kinds arrayed in highly specific sequential order in paired complementary strands helically coiled about each other, came the discovery that the information contained in the linear sequential array of codons is read out by the formation, under the influence of polymerase enzymes, of RNA, which, as a "messenger," transfers the information to the synthetic centers of the cell—that is, the ribosomes. There, with the aid of smaller RNA molecules, amino acids are joined into polypeptide strands having the specific amino acid sequence dictated by the RNA transcript of the DNA code. The synthesized protein is utilized intracellularly or exported for extracellular function, but some of the synthesized protein molecules may manifest an affinity for the DNA, thus constituting a negative-feedback control capable of repressing further readout at particular parts of the DNA concerned. Other proteins, histones, and macromolecular substances may similarly repress DNA readout. Hormones and small-molecule metabolites may play a similar role, either directly or, more often, by interacting with and changing tertiary conformation of repressors. Allosteric modulation of repressor molecules probably plays a major role in adapting genetic function to physiological needs at each particular time and place during the development and life of the organism.

Basic to the process of the switching on or off of gene action is the phenomenon of *molecular recognition of specific sites* along the monotonic sequence of nucleotide bases where binding of the repressor—possibly a protein, or other substance—has high stability. The hypothesis of gene control which now dominates molecular genetics must be viewed primarily as descriptive phenomenology made possible by the application of biophysical and biochemical methods to microbiological and cellular problems. Pending the developments of a rigorous theory of protein-DNA and protein-RNA interaction, knowledge of even the primary genetic process—to say nothing of the more complex epigenetic processes that adapt intracellular syntheses at each locus to developmental and physiological needs—must, unfortunately, remain at a phenomenological and empirical level. We still lack a comprehensive and consistent body of theoretical biophysical chemistry comparable in force and power with that of theoretical physics.

Immunological processes also depend on molecular recognition, in this case recognition of the antigen or other immunogen by a specific protein—gamma globulin—elaborated by blood-borne immunocytes (plasma cells, lymphocytes) which are the vertebrates' first line of defense against alien macromolecules. The immunization process involves plastic alterations of the chemical intelligence-service of the organism, so that alien molecules can be recognized by gamma-globulin molecules, which, after combining with the alien molecules, presumably consign them to metabolic dismemberment.

In but a few decades, immunization techniques have practically eliminated many infectious diseases and have shifted the emphasis of medical research to the area of the so-called degenerative and geriatric diseases. Basic knowledge of the immunological processes is, however, still largely lacking. Even less is known, in rigorous chemical theory, about the mechanisms by which the molecular intelligence system of the body—the immunocytes and their molecular effectors, gamma-globulin molecules—rec-

ognizes the selfness of the individual and rejects surgical grafts, even from siblings. Here the component-system, molecule-organism impasse is prototypically displayed. How can *my* gamma-globulin molecules express the individuality of the entity *me* as compared with *your* gamma-globulin molecules, which express and recognize the individuality of the entity *you*? No physical test or system yet devised can make such distinctions, only living cells and organisms.

Despite the profound ignorance of the physical basis of this cellular and molecular recognition phenomemon, it has been found that administration of cytotoxic drugs represses molecular selfness recognition, permitting the successful grafting of organs such as kidneys not only from human nonrelatives but even from infrahuman donors. Even at the highly empirical level at which transplantation programs are currently based, clinically rewarding progress in transplantation is being made. This is perhaps a portent of things to come if similar empirical attempts are made in the application of molecular science to the neurological and behavioral diseases.

I shall shortly consider whether the phenomenon of molecular recognition, basic to the processing of genetic and immunological information, also holds the key to an understanding of memory, learning, and other mental processes in man.

Behavioral Sciences and Neurosciences in Today's Culture

A striking parallel exists between the bustling activity in biology in the decade or two after the turn of the century and the quickening in the neurological and behavioral sciences today. There is the same preoccupation with defending and conserving the insights of an earlier generation (for example, those of Pavlov, Cajal, Freud, and Sherrington) in a period characterized by explosive advances in molecular biology. This preoccupation tends to inhibit original advances which might alleviate the mental diseases that now fill more hospital beds than do all other diseases combined. Today, tensions far exceeding those which characterized the molecule-chromosome-organism impasse of the 1920's result from a variant of the component-system dilemma which may be described as the molecule-brain-mind impasse.

Today in the behavioral sciences there is intense activity in studies of perception, drives, conditioning, memory, sleep, wakefulness, awareness, attention, consciousness, emotion, pathological states (neuroses, psychoses), and so on. And there is great need for these investigations, for through them may come practical methods not only of alleviating mental disease but also of improving learning ability—a necessity for the disadvantaged members of the world population.

Behavioral studies are highly phenomenological in nature. Some investigators who are by no means naive in their experimental logic or unsuccessful in achieving useful results manifest almost studied indifference to brain mechanisms—to what is inside the "black box"; rather, concern is chiefly with empirical relations between environmental factors and the organism's response to these factors.

This year marks the centennial of Mendel's discoveries. Though we may hope for a modern Mendel to correlate memory and learning parameters in man with some simple function of biological or physicochemical variables, unfortunately the complexity of the phenomenology of behavior makes the prognosis for such an eventuality poor.

Neurological fields, including neurophysiology, neuroanatomy, and neuropathology, are also in a healthy state of expansion. Since the discovery of the ongoing rhythmic electrical activity of the brain ("brain waves"), indicating constant high dynamic activity, many correlations with functions have been established. Anatomical and electrophysiological correlates of psychological states, including the highly significant process of conditioning in learning, have been effectively studied. Elegant demonstrations have been made of hierarchical levels of "wired-in" neuronal nets subserving sensory function; the work of Hubel and Wiesel (*5*) and of Lettvin, Maturano, and their associates (*6*) springs instantly to mind. There is healthy debate between, on the one hand, proponents of electropositivism, of the "wired-in" and of the probabilistic variety, and, on the other hand, the molecular neurologists, who are inclined to see dynamically and specifically responding molecular switchgear as the prime operators in the permanent storage, transfer, and fast retrieval of experiential information in the brain.

Detailed knowledge of neuronal circuitry on the part of the neurosurgeon is vitally important to a patient requiring exact localization of a lesion in his central nervous system. And application of high-resolution electron microscopy in studying serial thin sections of brain, especially of the previously refractory neuropil areas, promises eventually to require rewriting of the neuroanatomy books. Even if it were possible to specify in detail the neuronal interconnections in the human brain, the complexity would be so great as to defy human conceptualization, possibly even when aided by mathematical and machine computational facilities.

In neurobiology the dynamic approach initiated by Paul Weiss (*7*) and others shows that the neuron is not the static cellular entity portrayed by textbooks but is in a constant, high steady-state metabolic activity, synthesizing neuroplasm which moves down and perhaps undergoes cyclosis within axonal regions (*8*). These discoveries open up new avenues of exciting research which complement the new dynamism of neurochemical research: the investigation of protein synthesis and coding control responsible for neurophysiological function.

The Neurosciences Research Program

It is evident from the foregoing brief sketch that today, as at the turn of the century, behavioral sciences and neurosciences are characterized by fruitful developments in many directions. Does our present-day period have tensions resulting from the need to formulate satisfying concepts of the physical basis, not of how protoplasm manifests life, but of how the nervous system manifests memory, learning, and other mental processes in man? The molecule-brain and the brain-mind dilemmas are formidable conceptual barriers to progress. Because the intellectual impasse concerns man's own nature, the seriousness of the tension is multiplied. Merely to achieve a comprehensive overview of the problem in all its dimensions, while avoiding the self-defeating roadblocks erected by traditional professionalism and "discipline"-bound parochialism, requires more than can be accomplished by any one individual. A new

idiom in investigation, synthesis, and communication is needed. It was with this in mind that, 3 years ago, a group of creative scientists from various disciplines and from many universities here and abroad voluntarily organized, under the sponsorship of Massachusetts Institute of Technology, the Neurosciences Research Program. The "faculty" of this invisible college is aided by a highly competent full-time staff operating in a well-appointed center located in the House of the American Academy of Arts and Sciences. From evidence so far gathered it seems that, without reduction of the intensity of research and education in the more classical behavioral and neurophysiological sciences, new breakthroughs may be expected from application of insights gained from the recent explosive advances in molecular genetics and molecular immunology; new fields of molecular neurology and of molecular neuropsychology are in the making.

Molecular Neurology and Molecular Neuropsychology

Perhaps the most compelling neurobiological evidence for the primary role of molecular recognition of coded information stored in macromolecules of the brain cells is the formation of the fantastically complex neuronal network of the human brain in embryogenesis and development. Even if it is assumed that the specific readout of DNA occurs in the 10 billion neurons of the brain—readout capable of producing all the myriad kinds and shapes of neurons which compose the circuits of the human brain—it is still necessary to account for the proper synaptic connections of the numerous terminals. No microworkmen make the requisite connections at synapses according to some preordained blueprint or wiring diagram. Rather, it is suspected, the blueprint is expressed in the protein-protein molecular recognition in the membranes of the axonal terminals and dendritic receptors, whose number is legion. Only molecular identification tags could accomplish this fantastic biogenetic feat.

The most compelling evidence linking molecular recognition of the DNA-RNA-protein type with behavior is the fact that some of the most complex behavioral patterns observed by naturalists are innate or instinctual in type. These are not learned from information received experientially; hence they must, by definition, derive from the operation of the DNA-RNA-protein genetic molecular recognition system. However, the complexity of such a system, with its specificity of temporal sequencing and spatial patterning, defies comprehension by the human mind.

Equally staggering is the process which, over the years, stores an almost infinite number of memories and permits their retrieval in conscious (or unconscious) recall. The great psychobiologist Lashley sought to identify the memory trace, or "engram," as he called it (*9*). Is it to be found in specific neuronal circuitry? In molecular specificity? Is it permanently localized in the neurons involved in the sensory processing over which the information originally arrived?

Because individual neurons may receive synaptic inputs upon their dendritic trees from thousands of other neurons and because their axons may, in turn, make synaptic connections with dozens or hundreds of other neurons, specific nets could be determined and perpetuated as engrams only by subcellular, probably molecular, switchgear. It seems profitable to postulate that protein-protein molecular recognition is function-determinative at junctions between neurons in nets, in a process analogous to antigen-antibody interaction, and that such recognition may carry out engrammatic as well as structural instructions. Electron-microscopic evidence suggests that transsynaptic structures, in certain cases, may bridge the cleft between pre- and postsynaptic membranes and may effect such a protein-molecular switchgear function (*10*).

Antisera prepared against a purified protein, thought by B. W. Moore (*11*) to be characteristic of all vertebrate brains (and of no other tissue), cross-react with immunogens of all vertebrate brains studied, suggesting remarkable evolutionary stability of this protein (*12*). It may be a member of a family of acidic proteins one or more of which may function as the molecular effector of neuronal recognition, much as gamma globulin functions as the molecular recognition protein of vertebrate immunocytes (*13*), but which evolved long before the effectors of immunology did, in answer to the need of invertebrates for neural coordination and analyzer-integrator systems to subserve memory and learning.

Specific proteins stimulate the growth of certain neurons—for example, those of the sympathetic nervous system—presumably by switching on genes necessary for the synthesis of proteins characteristic of these neurons in morphogenesis (*14*). Possibly it is the proteins of the somatic or neuronal *target* cells destined to be innervated by, or synaptically interconnected with, the outgrowing neurons that switch on the genes necessary to stimulate neuronal growth. If the axonal outgrowth in the direction of the source of the diffusing protein evocator predominates, the appropriate connectivity will be favored.

Antibodies directed against this nerve-growth factor inhibit the growth of sympathetic neurons and, if applied during development, prevent the formation of a functional sympathetic nervous system.

Another crucial line of evidence for the role of molecular recognition in brain function is represented by the remarkable experiments of Mihailović and Janković (*15*). They have developed antisera against particular portions of the cat brain, such as the caudate nucleus and the hippocampus, by injecting into rabbits macerated caudate and hippocampus material that had been carefully dissected from cat brains.

When such antisera are introduced into the ventricles of cat brains in various parts of which electrodes have been implanted, only the caudate or hippocampal regions fail to respond electrically; this "silence" suggests that the antibiodies, by combining with these specific regions, have abolished function in them. Behavioral correlates of such immunological ablation have also been observed. This technique, after much elaboration and critical assessment, may provide a valuable tool for determining the function of particular brain parts and conceivably may become an adjunct to neurosurgery in human neuropathology. The application, in developing animals, of antisera against specific parts of the brain may result in animals lacking these regions in their brains; these might be of help to experimenters in determining the function of the immunoembryologically ablated parts.

In the context of their application in the rapidly expanding fields of molecular neurology and molecular neuropsychology, recent techniques and concepts of neuro- and psychopharmacology offer much promise. Affective behavior, involving mood and coloring of normal behavior or various degrees of abnormal behavior, is thought to be conditioned by small-molecular modulators, such as the potent biogenic amines and a wide variety of pharmacological agents, including tranquilizers. Such compounds may affect not only axons and synapses in the central nervous system but also the nerve cell bodies, possibly by influencing synthetic processes through their biogenetic controls. Such experiments may lead to a better understanding of the nature of brain and behavioral abnormalities and, hopefully, to methods of improving normal memory processing and learning.

A Protocol for Progress

Man is distinguished from all other animals by his ability to transduce what goes on in the billions of neurons and across many more billions of synaptic connections in his brain into written, spoken, and graphic symbols. When the skillful manipulation of mathematical symbols was added to the list, science as we know it was born. Development of computer science and of other symbol-processing prosthetic devices further expands man's ability to react adaptively to his environment and indeed to control it. A substantial increase in his understanding of these processes, physical and phenomenological, would lead to a new science literally unimaginable in terms of present knowledge.

Whether one likes it or not, man has embarked on the greatest of human experiments, probably far overshadowing in potentialities the exploration of outer space—namely, that of determining whether, by taking thought, man can discover the mechanism of thinking and whether, by so doing, he can achieve new orders of understanding not only of the universe about him but even of the dimensions of his own nature, not excluding certain aspects of his inner personal nature.

Any activity of such a character will necessarily have aspects of risk, will have negative as well as positive potentialities. But as President Julius Stratton (*16*) pointed out in his inaugural address at Massachusetts Institute of Technology, in connection with problems associated with atomic energy, such risks must be taken. Scientific inquiry cannot be stopped by society's ideas about the real or imaginary consequences of the inquiry; rather, society must learn to deal adaptively and constructively with advances in knowledge, particularly in knowledge about man's own nature.

Some negative aspects, where predictable, should prove avoidable. Immunological science and technology has not only revolutionized medicine, with the result that man's life span has greatly lengthened, but has also posed certain socioeconomic problems. Now, daring application of poorly understood techniques has initiated an era of organ transplantation which further complicates medical and socioeconomic problems. Similar headlong and widespread technical development in the neurosciences before substantial scientific understanding is attained could catapult society prematurely into forensic problems of public health and welfare, requiring social, economic, legal, moral, and perhaps religious judgments, compared with which the problems of fluoridizing public drinking water and legalizing the dispensation of contraceptive pills would pale into insignificance.

Still graver consequences must be considered, which follow from the fact that the absolute weapon—a term acceptable only to the military mind —is not an atomic missile but the human mind which conceives and devises such weapons.

We must hope that development of the positive, constructive aspects, by outstripping the negative ones, will convince men to choose cultural evolution rather than willful annihilation. In contrast with the eon-slow pace of evolution, the pace of psychological or cultural evolution is proceeding with a dizzying, autocatalytic acceleration: man's genius is ever evolving new ways to evolve more rapidly.

We come at last to the most deep-seated cause for tension, experienced not merely by behavioral scientists and neuroscientists, or by scientists generally, but also by philosophers, theologians, and indeed by all thinking men from time immemorial. It stems from the brain-mind conceptual impasse, from the ontological problem which Tillich identifies as man's deep concern about the nature or ground of his being. Are the issues raised in my previous discussion relevant to these concerns?

Scientific concepts may, in themselves, reflect great intrinsic beauty. But, as Polanyi (*17*) has suggested, it may never be possible through methods of physical science to represent life or mind in terms of a machine or a neural model, to symbolize or encode man's personal knowledge, his deepest concerns, and his inner apprehension of reality. Man strives to express these personal qualities with the metaphoric help of poetry, music, and the plastic arts, as has been stirringly attested by Paul Horgan (*18*) of Wesleyan University, who poignantly identifies the root of the matter: man and his mind or spirit constitute an entity whose awareness of itself and of reality—hence his ability to convey this knowledge through scientific, literary, or artistic symbolism—is personal, quantal, and nonfragmentable.

Behavioral scientists and neuroscientists find it premature, inconvenient, or even meaningless to address themselves to the problem of human selfhood as a psychological entity. Is there such an entity, or, on the contrary, is consciousness fully accounted for by the firing of billions of neurons in countless circuits? There are twice as many neurons in one human cortex as there are inhabitants on this planet. If each inhabitant had a private telephone wired for communication with a substantial fraction of all the other inhabitants and used it constantly, would such global intercommunication constitute an emergent global entity having properties in any way comparable to self-awareness and consciousness in human beings? I doubt it!

Molecular neurologists understandably may demand immunity from discussion of such subjects while they get on with their reductionist job. However, new insights may come from investigations of the physical properties of network systems of biomolecular polymers—not merely linear polymers like DNA, but giant networks of interbonded polymers—capable of subserving properties emergent from the combination of the inexhaustible repertoire of macromolecular parameters with the cybernetic, closed systems-

nature of networks. Might such a system prove useful as a model of ongoing fast-processing of information in the brain?

Molecular neurology, which is already on the way to becoming firmly established, together with molecular neuropsychology, which is emerging as a coherent field, seems destined to provide a powerful thrust in modern science. Society may well encourage, indeed demand, full speed ahead in these fields because of their important bearing on mental health, on the understanding of mechanisms of memory, learning, and other psychological parameters basic to science itself, and on man's deep personal concern about the nature of his being.

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Electrophoretic Variation in Enzymes

Mutations which do not alter catalytic activity provide a major tool for biochemistry and genetics.

Charles R. Shaw

That genetic variation may be expressed through altered enzyme activity has been appreciated since Garrod developed the concept of inborn errors of metabolism. The concept was expanded and clarified concurrently with the one-gene, one-enzyme hypothesis elaborated by a number of workers in biochemical genetics. In the past two decades, a large body of knowledge on genetic variation in enzymes, both in haploid and diploid organisms, has accumulated at an accelerating rate. Most of this information is based on alteration in catalytic activity of the enzyme.

Technical developments have recently made possible the high-resolution "zymogram" display of a number of enzymes from whole-tissue extracts. These new techniques utilize zone electrophoresis (*1*) followed by histochemical staining methods to demonstrate the zones of enzyme activity directly in the electrophoretic medium (*2*). An important application of the zymogram method has been the investigation of genetic alterations which change the electrophoretic mobility of enzyme molecules. The genetic variants disclosed by these methods are enzymes in which activity is retained but structure is presumably altered. This is in direct opposition to the earlier studies by which enzyme variation could be detected only as a change (including absence) of total catalytic activity.

A large and rapidly increasing number of electrophoretic variants of enzymes have been discovered, most of them in the past 3 years. Because many of these are mutants which produce no apparent change in the action of the gene product, they represent a unique and important parameter in biological research. It therefore seems worthwhile to review the types and frequencies of such enzyme variants and to consider their significance for genetics and biochemistry.

This discussion will be limited to diploid organisms. To consider diploids and haploids together is scarcely valid, for the two appear to represent totally different approaches to evolution. Regulation of their metabolism and growth is generally different (*3*), in that microorganisms have many inducible enzymes and flexible systems of gene control, whereas diploids probably have mainly constitutive enzymes, with control mediated by feedback mechanisms affecting the enzyme activity rather than gene activity. (Knowledge on this subject is only preliminary.) Diploids, moreover, reproducing mainly through sexual mechanisms, utilize the vastly greater flexibility and polymorphism resulting from sexual recombination and, having most genes present in duplicate, they can "experiment" with mutations while continuing to produce a normal product from the nonmutant allele.

Nature of Electrophoretic Variation

The now classic studies of Ingram and his co-workers (*4*) on genetic variants of human hemoglobin demonstrated that changes in electrophoretic behavior result from substitutions of single amino acids in the polypeptide chain. The altered electrophoretic mobility reflects a change in the net charge of the protein molecule which occurs when the amino acid carries a

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