Biochemical Actions of Hormones

VOLUME IV

Edited by GERALD LITWACK

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Preface

Because of the rapid pace of progress in the field of endocrinology, "Biochemical Actions of Hormones" will now be an open-ended treatise. This will enable us to record continuously major advances in the field so that the volumes can be referred to by researchers in endocrinology and related fields for focused information. We do not intend to make this an annual publication, but rather to produce volumes when progress merits it. The major theme of how hormones work will continue to be the thrust of the treatise.

A variety of subjects are covered which have not received emphasis in earlier volumes. Subjects bearing a direct relation to hormone action are affinity labeling, prostaglandins, regulation of protein turnover, control of cholesterol biosynthesis, the β-adrenergic receptor, hormone synthesis and release in pituitary cells in culture, the mode of action of insulin and androgens, and the roles of cyclic AMP in the central nervous system.

I had corresponded with Dr. Gordon M. Tomkins concerning a manuscript he was working on entitled The Origins of Hormones in which he was to give us some of his ideas on the encoding of signals into steroid molecules. Presumably, this would have followed the style of logic set for cyclic AMP and ppGpp in his paper entitled The Metabolic Code which was published in 1975 (*Science* **189**, 760–763). Perhaps he would have unveiled some real insights and working hypotheses for us. Gordon's untimely death prevented his thoughts from emerging in this volume. Consequently, we dedicate it to Gordon's memory. Most of the contributors to this volume knew Gordon personally, and all were enthusiastic about the dedication. I asked Dr. Bruce Ames to write a tribute to Gordon which appears at the beginning of the volume.

GERALD LITWACK

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Gordon M. Tomkins (1926–1975)

Bruce N. Ames

VITAE

Gordon Mayer Tomkins was born in Chicago on June 4, 1926 and grew up in Los Angeles. He came from a Jewish family. The two interests that played such an important role in his life stemmed from his family background—his father was a physician and endocrinologist and his mother is a musician. While still a teenager he not only finished college, graduating from the University of California, Los Angeles (cum laude) with a major in philosophy, but became an accomplished musician as well. He played classical music on the clarinet, and helped earn his way through college by playing the saxophone with a variety of well-known jazz and swing bands, including Stan Kenton and Charlie Barnett. In 1945, having just turned 19, he entered Medical School at the University of California, San Francisco. After 2 years he transferred to Harvard Medical School where he received his M.D. (cum laude) in 1949. He interned the following year at the Peter Bent Brigham Hospital in Boston. In 1950 he began his studies at the University of California at Berkeley for his Ph.D. degree with I. L. Chaikoff, which he completed in just 3 years. He did his thesis on the biosynthesis of cholesterol. While at Berkeley, Gordon married Millicent Hanson, a talented musician and painter. In 1953 he joined the National Institute of Arthritis and Metabolic Diseases in Bethesda, Maryland, where he remained for 16 years except for a sabbatical year in Paris in 1961–1962. He was Chief of the Laboratory of Molecular Biology from 1961 until he left NIH in 1969 to become Professor of Biochemistry at the University of California Medical School at San Francisco. Gordon died in New York City on July 22, 1975 of

complications following brain surgery for the removal of a tumor. In addition to his wife, he left two daughters (Tanya and Leslie), his mother, and his many friends. He had an extraordinary talent for making friends.

GORDON

Gordon's intellectual power was extraordinary: he was able to reach into remote corners of widely different fields and put together wonderful, coherent theories from assorted and apparently disparate facts. His interest was easily aroused and his mind was constantly at work, spanning vast knowledge and absorbing information like a sponge, spinning theories and conclusions in profusion. With the help of a photographic memory, he could recall old, relevant observations and cleverly fit them into ingenious patterns. His quick understanding made him an excellent sounding board for scientists (and people in other fields) in a variety of disciplines. When he would meet another scientist of comparable alertness, their conversation would go on in half sentences (all that was necessary to say) to the dismay of others present.

Gordon positively delighted in learning new areas in a truly professional way. When I first met him he did not yet know much about molecular genetics, but he was constantly asking me for details about progress in the area and was soon able to explain some of them to me. I still remember when he stopped me in the hall to discuss the new operon theory in the Jacob–Monod paper which he had just read in French (he spoke French fluently) in the latest *Comptes Rendus*. In recent years, when I had long despaired of keeping up with the baroque intricacies of lambda bacteriophage genetics, though I should have, Gordon would often point out some interesting new aspect of lambda which I should have known about, or which he thought was related in an interesting way to some hormonal function. In science, no area of biology or biochemistry was alien to him. I remember his learning X-ray crystallography from David Davies one year and his enthusiasm about the theoretical aspects of light scattering (arising from his glutamic dehydrogenase work) another. He always seemed to be testing his ability to master new fields and to keep abreast of whatever exciting was going on in science, or in other fields for that matter.

Gordon was a true intellectual with an extraordinary curiosity and knowledge of all aspects of human culture.

I remember, in our old group in Building 2 at NIH, the best of times were when we all were standing in the hall discussing some new theory with Gordon talking away, often while Gordon's secretary was vainly trying to get him off to some appointment (even two conflicting ones that he had agreed to absentmindedly: he never liked to say no to anyone). This intense involvement in science and with people was the main cause of his disorganized life style which all of his immediate colleagues and friends remember with amusement.

Certainly part of Gordon's intellectual power came from his phenomenal memory, which always amazed me. He could describe in great detail some movie of the 1940's which he had seen 20 years ago or remind me about some experiments I had published 10 years ago and had completely forgotten about. We had taken a trip to Europe (together with Jesse Rabinowitz) in the late 1950's and 15 years later Gordon could still recall all the marvelous details of the meal at Il Pappagallo or of whatever opera it was that we saw in Venice.

Because of his memory, breadth of knowledge, and enthusiasm for science he was the best companion to have at a scientific conference. I would soon start to drift off at a meeting and think about whatever idea was occupying me at the moment. Gordon followed everything and would give an incisive summary (with his own gloss) of all the interesting papers that I had missed.

Gordon had a special talent for making friends. His brilliance and breadth of knowledge were never intimidating; people who talked with him never came away feeling stupid. He had the capacity for making people feel that what they were doing was more exciting than they had thought before they spoke to him. He also charmed people with a marvelously wild sense of humor, and an incredible torrent of jokes poured forth continuously. His wit was sharp, but kind, and funny, without sarcasm. I guess that was due to the fact that he enjoyed life and loved people, and his jokes about both were never cutting, but perceptive and hilarious. Gordon was famous for his warmth and sympathy, understanding, and patience. He was always ready to stop what he was doing to listen and sympathize with anybody's problems, big or small. His warmth, sense of humor, vast intellectual power, depth of knowledge in so many areas, and proficiency in science and music, readily gained him a large number of friends in and out of science. Perhaps Gordon's most striking quality was his unusual ability to befriend people; his friendships endured and his friends loved him.

SCIENTIFIC WORK

Gordon was an extraordinarily productive scientist and published close to 200 papers in his 25-year career. His main scientific interest and contributions related to the mechanism of hormone action and metabolic control mechanisms, but he also made original and important contributions in a variety of areas of biochemistry and molecular biology.

Gordon's graduate work, completed in 1953, concerned the regulation of hepatic cholesterol synthesis. In these studies he demonstrated that an increased dietary cholesterol level inhibited the synthesis of the sterol from its precursors in the liver. This was one of the earliest examples discovered of negative feedback regulation of a metabolic process. As part of his work, he demonstrated that several cholesterol analogs could also function as inhibitors of sterol synthesis and suggested that such compounds might be used in clinical conditions characterized by an increased rate of cholesterol production. Several years later, this approach was adopted by various pharmaceutical concerns as a basis for the treatment of hypercholesterolemia and atherosclerosis.

After completing his graduate work he came to NIH and began studying the metabolic transformations of various steroids catalyzed by mammalian liver. He partially purified and characterized a 3β-hydroxysteroid dehydrogenase from liver and a series of enzymes which catalyze the sterospecific reduction of the 4–5 double bond of the A ring of a number of steroids. One group of these enzymes produced the 5α- and the other group the 5β-reduction products. During the course of this work, he found that separate proteins were required to catalyze the reduction of different steroids, even though their structures were very similar; for example, different enzymes were involved in the 5β-reduction of cortisone (11-keto) and cortisol (11-hydroxy). He even found evidence for reductases specific for hormone analogs not found in nature, such as the 9α-fluorosteroids. Work along these lines is still being done in other laboratories, and recently it was shown that 5α-reductase is the enzyme which converts testosterone to dihydrotestosterone. The importance of this is that testosterone was formerly thought to be the active male hormone, but recently it has been found that the dihydrotestosterone form is required for most of the biological activity.

By analogy with antibody-producing cells, Gordon proposed that the liver cell population was heterogeneous with respect to these

steroid-metabolizing enzymes (as well as other specific enzymes involved in the metabolism of foreign compounds) and, under his direction, a research fellow tested this hypothesis and obtained preliminary evidence confirming this theory [Bakemeier, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 379 (1961)].

Incidentally, I might mention that the poly(U) work, which resulted in breaking the genetic code, was done about this time (1961) by Marshall Nirenberg while working in Gordon's laboratory. Gordon had hired him as an independent, young associate.

Next, Gordon began to study the possible mechanisms of steroid hormone action and (together with K. L. Yielding) discovered that certain hormones were potent inhibitors of the electron-transport system in mitochondria. He traced their action to an inhibition of the amytal-sensitive portion of the NADH-cytochrome *c* reductase reaction and found that this inhibition was reversed by tocopherol and other lipids.

During the investigation of the inhibition of respiration by hormones he discovered that female sex hormones strongly inhibited the glutamate dehydrogenase reaction and that this inhibition could be reversed by ADP. Examination of the enzyme by analytical ultracentrifugation showed that the hormones induced disaggregation of the enzyme into subunits which was reversed by ADP. He, therefore, concluded that steroids and other small molecules could alter enzyme activity by inducing structural changes in the protein molecule.

The importance of this work is threefold. First, it was the first demonstration (pre-Monod by several years) that biological effectors could function by altering protein structure. Second, it showed that these alterations in structure could lead to changes in the catalytic capacity of an enzyme, not merely loss in enzyme activity but actually a qualitative change. Third, it suggested that steroid hormones themselves might function as allosteric effectors (more about this later).

He made several other contributions to the glutamic dehydrogenase field. In collaboration with E. Apella and Heini Eisenberg the correct structure of the enzyme was deduced despite the fact that a number of physical chemists had been struggling with the problem for a long time. They found that the active oligomer is made up of six identical subunits that aggregate in a unique, linear arrangement.

Gordon's original scheme relating the activity of the enzyme with its state of aggregation proved to be an oversimplification and has been revised to include another active component. However, his generalization that regulation of enzyme activity by small molecules operated through changes in the tertiary or quaternary structure of the

proteins has had an important influence in biochemistry and biology. These ideas were originally expressed in Gordon's publications on glutamate dehydrogenase in 1960, and were summarized in 1961 in his discussion at the Cold Spring Harbor Symposium that year. His contact with J. Monod at that conference, and during the following year (1961–1962) when Gordon was in Paris, was directly responsible for Monod's generalizations about allosteric proteins and biological regulation.

In the early 1960's Gordon and a group of his friends at NIH formed the Laboratory of Molecular Biology in the Arthritis Institute in Building 2. Gordon was Laboratory Chief and Gary Felsenfeld, David Davies, Martin Gellert, Todd Miles, Elizabeth Maxwell, Harvey Itano, Robert G. Martin, Michael Yarmolinsky, Giovanna Ferro-Luzzi Ames, and myself were among the scientists in the group.

At that time Gordon became interested in the cysteine biosynthetic pathway in *Salmonella* which was elucidated in collaboration with Nicholas Kredich (a postdoctoral fellow). This work corrected the theory proposed by Lynen that the immediate precursor of cysteine was pyruvate. They identified one of the two biosynthetic enzymes for cysteine with the *cysE* gene locus, purified to homogeneity both of the enzymes, and performed numerous physical chemical studies on them.

Other work in the early 1960's was stimulated by his stay in France and by some of our studies on polarity in bacterial operons. These were the experiments done by David Alpers showing that the expression of the *lac* operon was sequential, starting at the operator end. (This work was possible because he and Alpers and Appel had worked out a more sensitive transacetylase assay.) He did some further experiments on the mechanisms of expression of the *lac* operon showing that in the absence of protein synthesis the messenger could not be transcribed beyond the Z gene.

In 1966 he began the project which he continued until his death and which goes back to his first real love: hormonal regulation in mammalian cells. Len Garren, while working with Gordon as a postdoc, discovered that RNA synthesis appeared to be necessary for the *deinduction*, as well as the induction, of specific liver enzymes, a system responsive to the injection of hydrocortisone in rats. They called this phenomenon the "paradoxical" action of actinomycin D. In order to further investigate enzyme induction by hormones, Gordon, together with Brad Thompson, established the first cell culture system in which an enzyme was induced by a hormone. The enzyme is tyrosine aminotransferase and the hormones are the adrenal steroids. He

developed (with David Martin, Tom Gelehrter, and other associates) one of the best systems for the study of mammalian gene regulation. Tyrosine aminotransferase has become a well-developed model system in mammalian cells, largely as a result of his work. He and his co-workers used a number of biological techniques and performed chemical analyses of the various molecules involved in induction, including developing cell-free systems for the earliest and latest steps in hormone action. Work of Gordon (with John Baxter and other associates) supported the idea (also supported by other groups) that the steroids penetrate the cell membrane and associate with an allosteric protein, the specific steroid receptor. The steroids influence the equilibrium of this protein toward an active form which then migrates to the nucleus. A few minutes later, polyribosomes, actively synthesizing tryosine transaminase, appear in the cytoplasm. If the steroid is removed, the process is reversed. Interestingly enough, RNA synthesis is required to turn enzyme induction off as well as on. This is related to the same phenomenon that Gordon and co-workers had observed many years ago in intact animals. He proposed a model for regulation of the expression of the tyrosine aminotransferase gene (departing significantly from the Jacob–Monod scheme), and almost a hundred examples have appeared in the literature which can be explained according to his model. This work was one of the most extensive and best documented cases of hormone action and of the regulation of the expression of specific genes in mammalian cells. While studying tyrosine aminotransferase synthesis, Gordon and his students also started to study its degradation since the enzyme has a rapid turnover. They discovered that the degradation is controlled by mechanisms that seemed quite interesting, and while thinking about these processes they wandered into a somewhat different but closely related area: general cell regulation.

Gordon formulated a model called "pleiotypic control" in which he predicted that a "pleiotypic mediator," formed at the membrane in a number of cells, turns cell growth "off" by inhibiting membrane transport and macromolecular synthesis and stimulating protein degradation. This mediator was supposed to be more or less universally present in mammalian cells and its production or activity altered somehow in malignancy. He guessed that the mediator might have been ppGpp but then could not find any evidence for it. Later they came to the conclusion that cyclic AMP has all the properties of the supposed mediator. A number of predictions of the model and of the properties of the putative mediator have turned out to be correct. For example, malignant cells do not manufacture cyclic AMP under cir-

cumstances in which normal cells do (this work has been done in other laboratories including Ira Pastan's). He had written me a few years ago: "It seems to me as if we are close to some very basic control mechanisms of mammalian cells in which they are directed to stop growing and differentiate (by cyclic AMP) or to continue growing and not to express differentiated functions (cyclic GMP). This whole pleiotypic thing is still in a rather exploratory stage although I am quite excited about it."

From this great breadth of thought and experimentation in biochemistry and molecular biology, Gordon became convinced that beneath the biochemical complexity of eukaryotic cells lay relatively simple regulatory mechanisms. His fascination with that idea seemed to play a major role in the direction of his thinking, at the theoretical level as well as in the laboratory.

He understood the practical difficulties of correlating *in vitro* biochemical observations with *in vivo* processes in such complicated and poorly defined systems. Thus, he began to develop approaches to these problems which were more biological. Using a cultured cell line that responds to adrenal steroids or cAMP, Gordon and his colleagues isolated hundreds of clonal lines defective in the ability to respond to these agents. At the molecular level it was demonstrated that virtually all of the nonresponding variants carried lesions in various properties of their respective cytoplasmic binding proteins, either the steroid receptor or the cAMP-dependent protein kinase. (Among the co-workers involved in this work were Phil Coffino, Carol Sibley, and Keith Yamamoto.) An implication of these observations is that only a few general mechanisms govern a complex and disparate range of cellular processes. This novel genetic approach was an important advance which finally confirmed and extended a number of biochemical notions. Such systems are certain to play a central role in defining the components and reactions involved in the regulation of gene expression by small molecules.

At about the time that this aspect of his work was getting underway, Gordon began to formalize his thoughts concerning the evolution of intercellular communication. He noticed the intriguing fact that certain small molecules, cAMP among them, are not only themselves ubiquitous in nature but that remarkable preservation of the set of physiological states which they symbolize existed. He established a fresh view of the evolutionary importance of intracellular compounds which encode the metabolic status of a cell. From this followed the notion that hormones and neural transmitters arose to extend similar symbolism to communication *between* cells. Clearly, the capability for such communication was crucial to the evolution and survival of