Hormones and Cell Culture

BOOK A

Sato/Ross

Cold Spring Harbor Conferences on Cell Proliferation
Volume 6

Hormones and Cell Culture

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BOOK A








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Preface

Modern endocrinology and cell culture are relatively recent and contemporaneous developments. Cell culture is usually considered to have originated with the experiments of Ross Harrison in 1906. Although it is harder to fix a precise date for the origins of endocrinology, the word hormone was introduced by W. M. Bayless and E. H. Starling in 1904 as a unifying concept to denote internal secretions with regulatory function. For the most part the subsequent development of these two fields could have occurred in separate, hermetically sealed compartments. Early tissue culturists made sporadic attempts to see the effects of hormones on cultured cells. In several instances, a hormone was tested in culture shortly after its discovery. In the early 1920s, for example, George Gey reported positive effects of insulin on the proliferation of fibroblasts. However, despite these tentative moves to link cell culture with endocrinology, technology (until recently) has been lacking for a self-sustained movement in this direction. Even at the present time, most investigators utilizing cell cultures have taken their orientation from molecular biology and have sorely neglected the obvious opportunities relating animal physiology and cell culture. Similarly, professional endocrinologists have long disregarded the potential of culture techniques, perhaps originally because of the lack of differentiated cultures that retain the classical hormonal responses. Although such cultures have been in existence for some time now, most endocrinologists still view with suspicion any hormonal responses in culture. Prejudices die slowly. This meeting, held at Cold Spring Harbor Laboratory in August of 1978, may mark the beginning of a new era—an era in which explosive advances will be made in endocrine physiology using the techniques of cell culture.

In the early 1960s, Gordon Tomkins had already clearly envisaged the use of cell cultures and the modern techniques of microbial molecular genetics in working out the intimate details of the mechanisms of hormone action. What was visionary then is obvious today. Even the most classically oriented endocrine physiologist must admit that the mechanism of action of a hormone in an intact animal must correspond to any mechanism elucidated in cultured cells. However, when it comes to relating hormonal reactions in culture to higher levels of physiological organization, doubts will continue to

linger. For instance, are growth factors in fact hormones that lend themselves to discovery by culture techniques or are they in vitro artifacts? Time will ultimately settle this question.

For now, we can optimistically expect that culture systems, far from confusing the issues with meaningless artifacts, will turn up new facts not readily discoverable in any other way. As an example, it has recently been shown that serum can be replaced by mixtures of hormones in cell culture medium. GH_3, a pituitary cell line, requires insulin, transferrin, T_3, PTH, TRH, FGF, and somatomedin C for growth in the absence of serum. To demonstrate these dependencies by classical means, one would have to perform pancreatectomy, hepatectomy, thyroidectomy, parathyroidectomy, hypothalamectomy, and hypophysectomy—clearly impractical, if not impossible. The point here is that the most radical endocrine ablation possible is to remove serum from cells in culture; when this is done, hitherto unsuspected dependencies and responses are revealed. One can thus argue that by this means insulin and T_3 could have been discovered as growth factors in culture. Had they been discovered in this way, would they have been viewed as artifacts of culture? Probably, yes!

Furthermore, serum has been the common additive to culture medium to promote cell proliferation for most diploid cells, and conversely low serum concentrations have been used to induce cell quiescence. By definition, serum contains both plasma and platelet-derived molecules. Normally, cells in vivo would be exposed to the equivalent of serum only under special circumstances involving tissue injury and blood coagulation. In contrast, cells in vivo are usually exposed to plasma or a filtrate of plasma. Recent studies have shown that plasma or plasma-derived serum provides a means of obtaining quiescence for many cell populations, which can then be studied under more physiologic circumstances. These should permit analysis of the function of hormones in varying combinations and concentrations in plasma in culture in terms of their role in physiology and in disease in vivo.

The pace of culture work with hormones is increasing rapidly today. To organize a meeting on such a topic, in which major lines of endeavor are only now emerging, was difficult. At the outset, we decided to exclude studies of myeloid and erythroid cultures, on the grounds that these subjects were so large as to require a meeting exclusively devoted to them. Hepatotrophins were also not included in this symposium, since a recent CIBA foundation meeting adequately covered this subject. Even with the exclusion of large portions of the field, what remains is still overwhelming.

The meeting achieved several important goals. One of these was the gathering together of individuals from several disciplines including endocrinology, oncology, pathology, pharmacology, physiology, biochemistry, and various aspects of cell and molecular biology. The interactions and communication that occurred between these participants would have pleased Gordon Tomkins, to whom the meeting was dedicated. We beg the reader's forbearance and ask that he accept these proceedings as a useful compendium of information on a newly emerging field of inquiry.

This meeting was subsidized by the National Cancer Institute, National Institute on Aging, National Institute of Child Health and Human Development, National Institute of General Medical Science, Fogarty International Center, and the National Science Foundation.

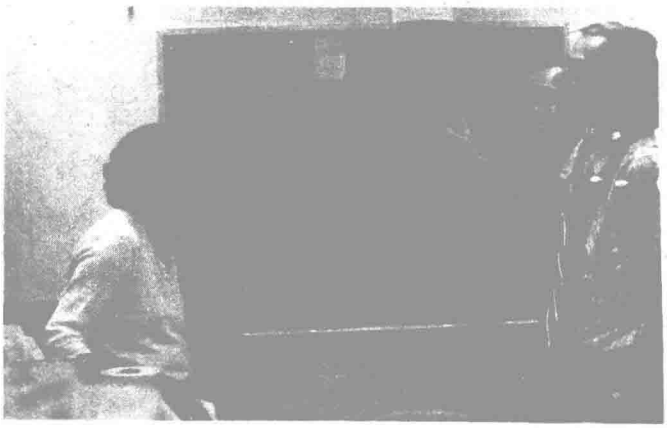
We wish to express our appreciation to Jim Watson for his many useful

suggestions and to Gladys Kist, Barbara Eggers, and Winifred Modzeleski of the Cold Spring Harbor meetings office for their help in organizing this meeting. We are also grateful to Nancy Ford (Director of Publications), Mary-Teresa Halpin, Chris Nolan, Roberta Salant, and Annette Zaninovic for their help in preparing these manuscripts for publication. Special thanks goes to Barbara Cowley-Durst, who was chief technical editor of these proceedings.

Gordon H. Sato
Russell Ross

(Clockwise) *Gordon Tomkins, 1927; with daughter, Leslie, in Bethesda, Maryland, 1960; interrupted rehearsal at Gif-sur-Yvette, France, 1961; with Ed Rall and daughter, Tanya, in France, 1961; relaxing at piano, 1975; with Giovanna Ames, in Mill Valley, California, 1970*

(Clockwise) *Dan Koshland and Gordon Tomkins, at Lake Tahoe, 1975; Tomkins and family, Greece, 1973; Tomkins, Steve Higgins, and John Baxter, University of California, San Francisco, 1974; with daughter, Leslie, in Mill Valley, California, 1972; speaking in Tokyo, 1974; Tomkins and wife, Millicent, at Mount Tamalpais, California, 1975*



In Memoriam

Gordon Mayer Tomkins (1926–1975)

As most scientists know, a scientific paper is usually a historical fiction. The scientific paper does not and, for the sake of efficient information transfer, cannot present the actual chain of events by which the author came to do his work. The results are simply embedded in a linear, logical narrative, instead of figuring as way-stations in an account of a zig-zagging, back-tracking path with fits and starts, guided by false leads and illogical, intuitive hunches. Moreover, the references cited in the paper usually do not render a true account of the persons that actually influenced the author; often the author is not fully aware of the real source of his ideas, although he does know that he has not actually read through most of the papers that he has cited. It is this aspect of the literature of science that makes the work of the historian of science so difficult. Since most of the essential information regarding interpersonal relations is missing from the literature, one cannot reconstruct from it the true causal chain that led to some important scientific advance, say the structure of DNA or the operon model of gene regulation. Accordingly, historians of science must try to gain access to the missing information via personal interviews, private correspondence, and autobiographical writings. Where this material is taken into account, it usually emerges that a rather small number of individuals had an influence on the history of a particular development that was enormously greater than would be inferred from the frequency with which their names appear in citations. Some of these key figures hidden in the historical background produced a crucial idea or discovery that brought an entirely new perspective to a discipline. Yet others wielded their disproportionate influence, not via any particular, readily identifiable epic contribution, but by dint of their fertile imagination and charismatic personality. Gordon Tomkins, to whose memory this conference is dedicated, was one of the second kind of seminal scientists.

Although Tomkins' name was, of course, well known to his fellow biochemists and molecular biologists, the enormous influence he exerted on the research and careers of many of them would never be suspected by, for

instance, a biochemist in Novosibirsk whose knowledge of the state of the art is based wholly on a study of original papers, reviews, and textbooks available in the Akademgorodok library. Our Siberian colleague could not know that Tomkins' extraordinary intellectual power allowed him to assimilate apparently disparate facts from widely different fields and assemble them into a coherent theoretical framework; that Tomkins had a vast knowledge and eerie capacity to recall diverse facts from his memory store whenever useful for supporting or rejecting some idea; that by combining an intense interest in science with an equally intense interest in people, Tomkins was the perfect partner for a creative discussion, in which he not only offered his own constructive contributions but also induced the other person to produce good ideas on his own; and, finally, that by being a positive thinker who was more interested in what was good in an idea than what was wrong with it, Tomkins radiated an upbeat feeling that inspired his colleagues to have faith in themselves and to carry on their projects with renewed enthusiasm. It was this power of positive thinking that probably accounted both for the amazingly large number of life-scientists who held Tomkins in affectionate regard (not to say, loved him) as well as for the disdain with which he was regarded by a number of biochemists. For it should be noted that Tomkins was by no means universally admired as a scientist and that he had many detractors.

Gordon Tomkins was born in Chicago in 1926 and spent his childhood and youth in Los Angeles. He received his undergraduate education at the University of California at Los Angeles, where he was awarded an A.B. in philosophy before he had reached the age of 20. By that time Tomkins had also become an accomplished wind-instrument musician, playing as a professional saxophonist with a number of well-known jazz bands, such as Stan Kenton's and Charlie Barnett's. Immediately after graduating from UCLA, he entered medical school and received his M.D. from Harvard at the age of 23. After a year's internship at the Peter Bent Brigham Hospital in Boston, Tomkins started graduate study in physiology at the University of California at Berkeley, working under I. L. Chaikoff. After obtaining his Ph.D. in 1953, Tomkins joined the staff of the National Institute of Arthritis and Metabolic Diseases in Bethesda, where he was to remain for the next 16 years. In 1969, at the age of 43, Tomkins was appointed Professor of Biology at the University of California Medical School at San Francisco. Although his own professional work lay wholly in the domain of basic science, Tomkins maintained a lasting concern for the implications of biological research for medicine. Both in Bethesda and in San Francisco Tomkins occasionally accompanied his colleagues on their hospital rounds. His comments on the rounds were well known to be eye-openers for the attending staff, house officers, and students, revealing that despite his apparent preoccupation with fundamentals, Tomkins had retained a profound grasp of medicine. Tomkins died in New York City on July 22, 1975, from complications following brain surgery for the removal of a tumor.

Tomkins' earliest studies in Chaikoff's laboratory were devoted to steroid metabolism. For his doctoral thesis research Tomkins had investigated the biosynthesis and degradation of cholesterol, as well as its conversion to steroid hormones. This work led to the elucidation of several crucial metabolic steps, especially those representing dehydrogenation reactions. It also aroused in Tomkins the lasting interest in the mechanism of action of

steroid hormones that was to be the *Leitmotif* of his whole later research. This interest, and in particular his encounter as a student with dehydrogenation reactions, led him to the study of the regulation of the function of glutamic dehydrogenase. The quaternary structure of this enzyme consists of several polypeptide subunits, and Tomkins found that estrogenic steroid hormones induce the dissociation of the enzyme into its subunits. Moreover, he observed that this hormone-induced dissociation of the enzyme is accompanied not only by a decrease in the glutamic dehydrogenase activity but also by an increase in alanine dehydrogenase activity. From these findings he derived the notion that hormones produce their effect by altering the structure of the target proteins with which they interact. Tomkins did not hesitate to extend this idea to regulatory ligands in general and, in 1961, proposed that "it seems quite possible that similar mechanisms might operate in the regulation of other systems, such as feedback inhibition in microorganisms, and even, perhaps, in induction and repression of enzyme synthesis." Thus he had begun to think of the regulatory significance of allosteric protein transformations before the term "allostery" had been put forward by Jacques Monod and his colleagues. Tomkins believed, in fact, that his presentation of the glutamic dehydrogenase findings at the 1961 Cold Spring Harbor Symposium had provided an important stimulus for Monod to formulate the general allostery concept. It was also during this time that Marshall Nirenberg, whom Tomkins had recruited for his section at the National Institutes of Health, carried out the experiments that led to the deciphering of the genetic code. According to Tomkins' recollection, he had suggested to Nirenberg's coworker, H. Matthei, that he add the homopolymer polyuridylic acid as a "control" to Nirenberg's mRNA-dependent cell-free protein synthesizing system. Tomkins reasoned that if the polyuridylic acid "control" stimulated incorporation of amino acids into protein, then the stimulation produced by natural mRNA in Nirenberg's system might be artifactual rather than informational. Matthei carried out this control, and the well-known result and its eventual interpretation became a major milestone in the history of modern biology.

Following the discovery of the allosteric regulation of glutamic dehydrogenase by steroids—possibly the most important discovery of his career—Tomkins spent a sabbatical year in Paris. There he interacted with Monod, François Jacob, and their coterie of brilliant molecular biologists that made the Paris of the 1960s the world center for the study of enzyme regulation. For Tomkins, perhaps the most important lesson of this Parisian sojourn was the power of genetics for unravelling the knotty problems of metabolic regulation. Subsequent work on the induction of bacterial enzymes in Tomkins' Bethesda laboratory was a direct consequence of this lesson. However, despite the rapid advances that were being made in the 1960s in the understanding of the regulation of gene expression in bacteria (and the contemporaneous high fashion of that field), Tomkins' main interest remained in regulatory phenomena in animal tissues. By that time it had emerged from a variety of studies that in animals steroid hormones can induce the formation of specific enzymes. Tomkins thought that this phenomenon is analogous (or maybe even homologous) to enzyme induction in bacteria and that, therefore, both methodology and ideology of prokaryotic gene regulation might be applicable to eukaryotes. Accordingly, he began to study the influence of steroid hormones on the formation of several